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Effect of Humoral and Tissue Factors on Insulin Action

James B. Field, M.D., Bethesda

Although the insulin requirement of the pancreatectomized human is approximately 40 units/day many diabetic patients have daily insulin dosages exceeding this amount.[1] In such cases absolute insulin lack would appear to be an inadequate explanation. Despite an insulin dosage greater than 40 units/day, a diabetic is not usually considered insulin resistant until his daily dosage exceeds 200 units.[2] Furthermore, during diabetic acidosis and infections a temporary resistance to the action of insulin is often observed.[3] In addition, the diabetes which accompanies acromegaly and Cushing's disease is frequently characterized by some degree of insulin insensitivity.[5] Even more puzzling is the labile juvenile diabetic patient whose blood sugar can show tremendous variations despite constant diet, insulin and approximately the same amount of exercise. The present paper concerns a study of some of the factors that might influence the effective insulin concentration reaching peripheral tissues and thus might explain some of the above mentioned clinical situations. The existence of circulating insulin inhibitors in some of these conditions has previously been demonstrated.[4-7]

METHODS

The method used for determining the presence in plasma of an antagonist to insulin has been described previously.[7] Briefly, it is based on the ability of insulin to stimulate glucose uptake by the isolated rat diaphragm in the presence of normal plasma. The increased glucose uptake resulting from exposure to insulin is referred to as the insulin effect and is expressed as milligrams of glucose disappearing from the incubation medium/gram of diaphragm. The absence or significant reduction of the insulin effect when test plasma was used has been taken as evidence for the existence of an insulin antagonist in that plasma.

The responsiveness of leucocytes to insulin was measured according to the method of Weinberg.[10] All glassware had been siliconized previously. Blood obtained in the fasting state was added to an equal volume of 3 per cent fibrinogen in Krebs-Ringer bicarbonate buffer. After sedimentation for thirty minutes the leucocyte-rich supernatant was carefully removed and gently mixed. Five milliliter aliquots of the leucocyte suspension were added to flasks containing either 1.5 ml. of Krebs-Ringer bicarbonate buffer, 7.5 mg. of glucose and 2 units of insulin or the Krebs-Ringer bicarbonate with glucose but no insulin. After a ninety-minute incubation period, the glucose remaining in each flask was determined. The results are expressed as milligrams of glucose disappearing from the incubation flasks per 10^{30} leucocytes. Both steroid treated normal individuals and rheumatoid arthritic patients receiving steroids were studied. Determinations when possible were done prior to and during the institution of steroid therapy so the patient could serve as his own control.

RESULTS

Table 1 indicates that plasma obtained from each of six chronically insulin-resistant patients contained an insulin antagonist. In some of these cases antagonist was still demonstrable when plasma was diluted 1:10. In the presence of normal plasma the insulin effect was 2.66 mg. of glucose/gram of muscle and this was not significantly altered by a tenfold dilution of the plasma.

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From Clinical Endocrinology Branch, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda 14, Maryland.

In general there was no good correlation between the titer of the insulin antagonist and the patient's daily insulin dosage. This lack of correlation has been demonstrated previously when the insulin binding capacity of plasma from resistant patients was measured.[4] The existence of insulin antibodies in the plasma of all diabetic patients receiving insulin for several months has been amply demonstrated by Berson and his colleagues.[11] An excess of this insulin antibody can usually be detected in plasma obtained from most diabetics with chronic insulin resistance.[4,12] Although the augmented amounts of antibody present during chronic insulin resistance may well explain this phenomenon, studies attempting to relate this antibody to other clinical situations have been somewhat disappointing.[13,14] Kalant was unable to correlate antibody titer and insulin sensitivity as measured by the glucose-insulin tolerance test.[13] Skom and Talmadge reported several diabetics in whom the antibody titer was rising at a time when the insulin requirement was falling, as well as the reverse situation.[14]

TABLE 1

Insulin effect in the presence of plasma from diabetics with chronic insulin resistance

Patient	Insulin dosage	Dilution of plasma	Insulin effect mg. glucose/gm. muscle*
Ov	400	Undiluted	0.20 ± 0.46 (6)
Bu	256	Undiluted	0.50 ± 0.77 (3)
Fr	220	Undiluted	0.08 ± 0.21 (3)
Th	250	1:10	0.50 ± 0.28 (3)
Ch	700	1:10	1.00 ± 0.75 (3)
St	580	1:10	0.19 ± 0.15 (3)
Normal	—	Undiluted	2.66 ± 0.18 (51)
Normal	—	1:10	2.51 ± 0.09 (89)

*Figures in parentheses represent the number of determinations with Standard Error of the Mean.

Steroid therapy has often been successful in the treatment of chronic insulin resistance.[15,16] Table 2 demonstrates that reduction of the insulin dosage as a result of steroid therapy in one patient is associated with a decrease in the effective titer of insulin antagonist. Before steroid therapy this patient's insulin requirement was 700 units/day and his plasma diluted 1:100 was still capable of inhibiting the insulin effect in the test system. Only when his plasma was diluted 1:1,000 was the insulin antagonist finally diluted out. Following successful therapy with 40 mg. of prednisone/day, his insulin requirement dropped to 120 units/day and antagonist could be demonstrated only when undiluted plasma was used.

Insulin antagonist was detected in the plasma of two of four new diabetic patients prior to insulin therapy (table 3).[7] Clinically there were no distinguishing features between the first two cases with antagonist and the last two without. They all had hyperglycemia, massive glycosuria and ketonuria without any evidence of diabetic acidosis. They all required insulin for the control of their diabetes and there was no gross difference in the initial insulin requirements of the two with antagonist as compared to the two without. Inhibitor could no longer be detected in the first two cases once the diabetes was controlled. This finding is consistent with the earlier observations of Vallance-Owen et al. that the insulin inhibitor present when the diabetes is uncontrolled is no longer detectable when the blood sugar is in the physiologic range.[5] More recently Vallance-Owen and his colleagues have reported that this inhibitor is also present in normal plasma but to a lesser degree.[17] When the plasma proteins were fractionated the inhibitor was in the albumin fraction. The material was not present in the plasma of a diabetic patient following hypophysectomy, suggesting that it might be of pituitary origin. At present it is not known what relation, if any, this inhibitor has to the one which has been described as

TABLE 2

Decrease in titer of insulin antagonist following steroid therapy in diabetic with chronic insulin resistance

Dilution of plasma	Insulin effect mg. glucose/gm. muscle Before steroids*	After steroids*
Undiluted	—	0.72 ± 0.67 (3)
1:10	0.85 ± 0.36 (3)	3.26 ± 0.32 (3)
1:100	0.33 ± 0.40 (3)	—
1:1,000	2.78 ± 0.62 (3)	—

*Figures in parentheses represent the number of determinations with Standard Error of the Mean.

TABLE 3

Insulin effect in the presence of plasma from new diabetics requiring insulin

Patient	Dilution of plasma	Before insulin Insulin effect* mg. glucose/gm. muscle	After insulin Insulin effect* mg. glucose/gm. muscle
Wh	Undiluted	0.32 ± 0.30 (3)	2.50 ± 0.60 (6)
Ka	Undiluted	1.16 ± 0.23 (3)	2.58 ± 0.44 (3)
Sh	Undiluted	2.36 ± 0.12 (3)	—
Or	Undiluted	2.27 ± 0.38 (3)	—
Normal	Undiluted	2.66 ± 0.18 (51)	—

*Figures in parentheses represent the number of determinations with Standard Error of the Mean.
From Field and Rigby, J. Endocrin. *19*:174, 1959.[7]

occurring in some patients during diabetic acidosis.[6] In this latter situation the antagonist was also evanescent, and it migrated with the α_1 globulins. Current methods of protein fractionation are not precise enough always to separate the α_1 globulins from albumin so it is quite possible that the antagonist during diabetic acidosis is the same as that described by Vallance-Owen and his colleagues.

The cause of the decreased insulin responsiveness of diabetics during infection is not fully known, although a role for increased adrenal activity has been postulated.[18] Table 4 indicates evidence for a circulating insulin antagonist in some diabetic patients during infections.[7] The first patient had a staphylococcal abscess and his plasma diluted 1:10 still inhibited the insulin effect. In this case the inhibitor had some of the characteristics of the antagonist present during diabetic acidosis in that it was still effective when the rat diaphragm was exposed to it prior to the addition of insulin.[7] The diabetes in the second case (St) was out of control as a result of severe infectious mononucleosis. Although the insulin effect using plasma from patient Os, with acute appendicitis, was lower than that observed in any normal plasma, it was not significantly decreased. Patient Na had acute *B*-coli pyelonephritis but her plasma did not inhibit the insulin effect. Further characterization of the antagonist in this situation has not been done, and it was not possible to retest plasma from these patients after the infection had been eradicated.

TABLE 4

Insulin effect in the presence of plasma from diabetics with infections

Patient	Dilution of plasma	Insulin effect mg. glucose/gm. muscle*
Wo	1:10	1.04 ± 0.47 (3)
St	Undiluted	1.10 ± 0.32 (6)
Os	Undiluted	1.56 ± 0.37 (3)
Na	Undiluted	2.07 ± 0.39 (6)
Normal	Undiluted	2.66 ± 0.18 (51)
Normal	1:10	2.51 ± 0.09 (89)

*Figures in parentheses represent the number of determinations with Standard Error of the Mean.
From Field and Rigby, J. Endocrin. *19*:174, 1959.[7]

There are also hormonal agents which modify insulin action, although the exact mechanism of their action remains to be elucidated.[19] Injection of growth hormone in suitable animals produces insulin resistance, and diaphragms removed from rats treated with growth hormone are less responsive to insulin than diaphragms from normal rats.[20] This finding suggests that the decreased sensitivity to insulin in diabetics with acromegaly might reside at the tissue level. However, plasma from some diabetics with acromegaly is capable of diminishing the effect of insulin on normal rat diaphragm (table 5).[7] Patient St was able to control her diabetes by diet therapy alone while all the other patients were receiving insulin therapy. Patient Wy, the only patient without insulin antagonist in her plasma, had received her usual morning insulin injection several hours before the blood was drawn for the test while in all the other cases blood was drawn twenty-four hours after the last insulin. It is possible that in Wy the previous insulin had saturated the antagonist so that none would be detected in the assay. It is not possible to tell whether the antagonist is growth hormone itself or whether it is related to previously mentioned inhibitors. The situation in regard to growth hormone is further complicated by observations that under certain circumstances this hormone may have a hypoglycemic effect.[21]

TABLE 5

Insulin effect in the presence of plasma from diabetics with acromegaly

Patient	Dilution of plasma	Insulin effect mg. glucose/gm. muscle*
Ma	Undiluted	0.97 ± 0.73 (3)
St	Undiluted	1.21 ± 0.25 (6)
Ja	Undiluted	—0.32 ± 0.35 (3)
Wy	Undiluted	2.28 ± 0.23 (3)
Normal	Undiluted	2.66 ± 0.18 (51)

*Figures in parentheses represent the number of determinations with Standard Error of the Mean.
From Field and Rigby, J. Endocrin. *19*:174, 1959.[7]

A direct antagonism between adrenal cortical steroids and insulin has not been demonstrated, although their influence on gluconeogenesis might tend to oppose the action of insulin. However, there is evidence for a further effect in respect to insulin action since diaphragms from cortisone treated rats are less sensitive to insulin than diaphragms from normal controls.[20] In addition, table 6 indicates that steroid administration to nondiabetic human beings decreases the responsiveness of their leucocytes to in vitro insulin stimulation.[23] During steroid therapy there is no apparent change in the basal glucose uptake of the leucocyte suspensions but there is a marked inhibition in the additional glucose uptake mediated by the addition of insulin (0.3 units/ml.).

It has not been possible to study these patients after steroid therapy to determine how long the effect persists. This inhibition of the insulin effect

resides in the cells rather than in the plasma since there was no evidence of an insulin inhibitor in the plasma. Thus the cause of the increased insulin requirements during periods of stress and in diabetes associated with Cushing's syndrome may reside partly in the tissue itself.

Epinephrine and glucagon may affect the peripheral action of insulin as a result of increasing the blood sugar.[19] In addition, epinephrine causes a decrease in the peripheral arteriovenous glucose difference[35] and antagonizes the effect of insulin on isolated muscle preparations.[34] Whether glucagon has a similar action is still a subject of controversy.[35]

TABLE 6

Effect of adrenal steroid therapy on insulin responsiveness of leucocytes

	Glucose uptake (mg.) by 10^{10} WBC					
	No therapy			Steroid therapy		
Patient	Basal	Insulin*	Per cent increase	Basal	Insulin	Per cent increase
C.K.	110	332	202	138	217	57
C.B.	106	294	177	110	171	56
Average four normals	119	294	147	—	—	—
R.B.	—	—	—	124	222	79
M.W.	—	—	—	118	206	74

*Insulin concentration 0.3 unit/ml.

Recent studies on the rate of absorption of insulin-I^{131} from subcutaneous and intramuscular injection sites have demonstrated moderate variation comparing one arm or leg to the other arm or leg in the same patient.[6,9] In general there was no relationship between daily insulin requirement and rate of insulin absorption. Insulin absorption rates in some chronically insulin resistant patients were similar to those found in nonresistant diabetics. Although these studies generally indicate that decreased effectiveness of insulin is not explained by local inactivation or delayed absorption, there is the occasional patient whose poor diabetic control results from repeatedly giving insulin injections into the same fibrofatty lump in the arm or leg. When the site of injection is varied in such a patient, much better control of diabetes is possible with smaller doses of insulin.

Several case reports have suggested that some cases of insulin resistance are due to failure of end organ response to the hormone.[26-28] We have recently studied a young diabetic patient who would seem to be an example of this phenomenon.[29] This patient had persistent hyperglycemia, glycosuria and intermittent acetonuria. She was completely resistant to insulin in doses as high as 38,000 units/day. Three months after her last known insulin injection it was possible to demonstrate large amounts of circulating insulin in her plasma, but no evidence for any insulin antagonist. Despite the increased amount of plasma insulin which was physiologically active in isolated rat diaphragm and intact mouse,[30] her blood sugar remained at hyperglycemic levels. It is possible that other diabetic patients also exhibit various degrees of tissue unresponsiveness at different times as an explanation for some of their lability in diabetes control.

SUMMARY

Various factors which might exert an effect on insulin action have been presented and discussed. Although in chronically insulin-resistant patients increased insulin antibody can usually be demonstrated, it is not known what role, if any, this antibody plays in the nonresistant diabetic. Successful steroid therapy of chronic insulin resistance is accompanied by a decrease in the effective titer of the insulin antibody. In some new untreated diabetic patients, an insulin inhibitor can be demonstrated in plasma. Control of the diabetes is associated with disappearance of the antagonist. Some diabetics with infections also have an insulin antagonist in their plasma as do some diabetics with acromegaly. At present the interrelationship and nature of these various plasma antagonists is not known. Leucocytes obtained from patients during steroid therapy are less responsive to insulin than leucocytes obtained before steroid administration, suggesting a direct steroid effect on peripheral tissue itself.

SUMMARIO IN INTERLINGUA

Le Effecto de Factores Humoral e Tissutal Super le Action de Insulina

Varie factores que exerce possibilemente un effecto super le action de insulina es presentate e discutite. Ben que in patientes qui es chronicamente insulino-resistente, anticorpore a insulina es usualmente demonstrabile in augmentate quantitates, il non es cognoscite qual rolo iste anticorpore ha in patientes non insulino-resistente o mesmo si illo ha ulle rolo del toto. Le successose tractamento steroidic de chronic resistentia a insulina es accompaniate de un reduction del efficace titro de anticorpore anti insulina. In certe nove e non ancora tractate diabeticos, un inhibitor de insulina pote esser demonstrate in le plasma. Le stabilisation de diabete es associate con le disparition del antagonista. Certe diabeticos con infectiones ha etiam un antagonista

contra insulina in lor plasma, e le mesmo es ver pro certe diabeticos con acromegalia. A iste tempore, le natura de iste varie antagonistas in le plasma e le relationes inter illos non es cognoscite. Leucocytos prendite ab patientes sub therapia steroidic es minus responsive a insulina que leucocytos prendite ante le administration de steroide. Isto suggere que il occurre un effecto directe del steroide super le tissu peripheric mesme.

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Special Article

Glucose Transport and Phosphorylation in Muscle of Diabetic Animals

C. R. Park, M.D., and H. E. Morgan, M.D., Nashville

It is generally thought that the impaired peripheral utilization of glucose in the diabetic animal is due in part to a depression of glucose uptake by muscle tissue. In the present paper, we shall attempt to analyze the disturbances in cell function causing this depression.

In a series of papers by Cori and his associates,[1-3] the proposal was developed that the low uptake was due to inhibition of the hexokinase system. The hexokinase reaction, in which glucose is phosphorylated to glucose-6-phosphate, was regarded as the first step in glucose metabolism and rate-limiting for glucose uptake by muscle. From studies with extracts from diabetic muscle,[2] it was proposed that the reaction was inhibited by pituitary and adrenal factors and that this inhibition was relieved by insulin. It was not possible, however, to obtain entirely satisfactory support for this theory. The inhibition was noted to be very unstable and could not be obtained with regularity. An effort by Stadie and associates[4,5] to duplicate the original observations did not succeed. Furthermore, it was not established experimentally that the hexokinase reaction was in fact the rate-limiting step for uptake by intact muscle, and subsequent studies showing the marked inhibitory effect of glucose-6-phosphate on hexokinase[6,7] have made it doubtful whether the enzyme was the site of inhibition in the original extract studies. Nevertheless, the "hexokinase theory" continued to receive serious attention, since many studies with intact tissue preparations indicated the presence of a metabolic block in the early phases of glucose metabolism at or near the level of the hexokinase reaction.[8]

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From the Department of Physiology, Vanderbilt University Medical School, Nashville, Tennessee.

Dr. Morgan is investigator of the Howard Hughes Institute for Medical Research.

Following the observations of Levine and associates,[9,10] a large body of evidence has been assembled to show that insulin accelerates the transport of glucose through the cell membrane of muscle.[8,11,12] Membrane transport has been shown to involve a specific interaction between glucose and a membrane component and to be a step in glucose metabolism that antecedes glucose phosphorylation.[12] In the diabetic muscle, it has been shown that transport is depressed[13,14] and is the major limiting step for glucose uptake.

While these studies established an action of insulin on transport, they did not exclude an additional effect of the hormone on phosphorylation as envisioned in the hexokinase concept. In this connection, recently developed experimental technics make it possible to distinguish effects on phosphorylation from effects on transport in intact tissues.[16-18] By use of these methods, it has been shown that phosphorylation is in fact depressed as well as transport.[19-21] Furthermore, the inhibition of phosphorylation appears to be due in part to pituitary and adrenal activity and can be relieved by insulin under certain conditions as will be seen below. In light of these observations, it would now appear that the disturbances in the diabetic muscle are best described by a concept which includes elements of both the transport and hexokinase theories.

Representative data that support this concept are shown in the table. These data are taken from studies of glucose uptake by the perfused, isolated rat heart. The methods employed and some of the results have been described in earlier publications,[12,21] and a full description will appear shortly.

In the heart from normal, fasted rats the glucose uptake is 4.7 mg./gm./hr. under the present experimental conditions. This value (experiment 1) is taken as a reference against which other rates are compared. As can be seen, no free glucose accumulates inside the cell during uptake. This is an important observation on which the following argument is based. Since the product

TABLE 1

The effect of insulin and pituitary and adrenal factors on glucose uptake by the isolated, perfused rat heart

Experiment number	Treatment of rats	Insulin in vitro 3 μg. per ml.	Glucose uptake mg. per gm. per hr.	Glucose uptake per cent of normal	Intracellular free glucose mg. per 100 ml.	Glucose phosphorylation mg. per gm. per hr.	Glucose phosphorylation per cent of normal capacity*
			Normal rats				
1	None	0	*4.7±0.2*	100	Ca.0	4.7±0.2	—
2	None	+	10.7±0.6	230	15±4	*10.4±0.6*	100
			Alloxan diabetic rats				
3	None	0	*2.0±0.3*	40	Ca.0	2.0±0.3	
4	None	+	3.5±0.3	70	58±3	*3.3±0.3*	30
5	Adrenalectomy	0	*2.0±0.3*	40	Ca.0	2.0±0.3	
6	Adrenalectomy	+	5.6±0.7	120	15±15	*5.5±0.7*	60
7	Hypophysectomy	0	*1.8±0.4*	40	Ca.0	1.8±0.4	
8	Hypophysectomy	+	7.6±0.5	160	26±7	*7.3±0.5*	70
9	Hypophysectomy and growth hormone	+	5.9±0.4	130	35±10	*5.7±0.4*	50
10	Hypophysectomy and hydrocortisone	+	4.6±0.5	100	39±10	*4.3±0.5*	40
11	Hypophysectomy, growth hormone and hydrocortisone	+	2.3±0.2	50	59±8	*1.8±0.2*	10
12	Insulin (24 hours)	+	*11.8±0.3*	250	Ca.0	11.8±0.3	>110
13	Insulin (24 hours) and growth hormone and hydrocortisone	+	*11.2±0.2*	240	Ca.0	11.2±0.2	>110
14	None	+ anoxia	*14.1±0.6*	300	Ca.0	14.1±0.6	>140

The glucose uptake values in italics provide measurements of the rate of unidirectional transport into the cell, since transport is the rate-limiting step for uptake in these experiments (see text). Uptake values not italicized indicate net transport rates only since free glucose is present inside the cell. The values in italics for phosphorylation provide estimates of the phosphorylation capacity. Values not in italics do not estimate the capacity, since the availability of substrate is restricted in these instances by the relatively slow rates of transport (see text).

All rats except those receiving insulin in vivo were fasted eighteen hours before removal of the heart. Diabetic rats were prepared by the intravenous injection of 6 mg. of alloxan per 100 gm. of body weight forty-eight hours or longer before use. Adrenalectomy was carried out four days and hypophysectomy three to six weeks before death. Growth hormone (a gift of the Endocrinology Study Section of the National Institutes of Health) was injected twenty-four hours and again twelve hours prior to sacrifice in a dosage of 100 μg./100 gm. Hydrocortisone was administered twenty-four, twelve and three hours before sacrifice in dosages of 2 mg./100 gm.

The perfusions were carried out for thirty minutes at 37° with 100 mg./100 ml. of glucose in oxygenated, bicarbonate buffer. Other procedures will be described in detail elsewhere.[23]

*These percentages take into account the variations in intracellular glucose concentration since they are referred to a curve obtained for phosphorylation in the normal heart as a function of intracellular glucose concentration.[23]

of transport into the cell is the free sugar, as shown in earlier studies,[12,13] the absence of free sugar indicates that glucose is phosphorylated as rapidly as it penetrates. As a consequence, the rate of phosphorylation is limited by the rate of transport. Transport is, in fact, the rate-limiting step for the over-all uptake of glucose, since it has been established that extracellular diffusion of sugars from the blood to the cell surface is relatively very fast.[21]

When insulin is added in vitro (experiment 2), glucose uptake is markedly stimulated and free glucose now is found inside the cell. This is interpreted to mean that transport is accelerated by the hormone to such an extent that the phosphorylation capacity is exceeded. Under these conditions, phosphorylation becomes the major limiting step for glucose uptake. It may be noted that the rate of phosphorylation is slightly less than the rate of uptake, the difference being due to the amount of free sugar accumulating inside the cell.

In hearts from severely alloxan-diabetic rats (experiment 3), uptake is reduced to 40 per cent of normal.

No free glucose is present in the cell, and transport remains the limiting step for uptake. Thus a low rate of transport must be the cause of the depressed uptake. When insulin is added (experiment 4), uptake rises, but the hormone effect is much smaller than in the normal tissue. There is, however, a very marked rise in intracellular free glucose indicating that transport into the cell has been accelerated. The failure of glucose uptake to rise to the normal extent under these conditions, where phosphorylation becomes the limiting step, provides evidence that phosphorylation is also inhibited. It is also clear that this inhibition is not relieved by insulin in vitro in the thirty-minute period of these experiments.

Further experiments show that adrenal and pituitary factors inhibit phosphorylation but not transport. In the absence of insulin, where uptake reflects the rate of transport, neither adrenalectomy nor hypophysectomy has any effect (experiments 5 and 7). On the other hand, in the presence of insulin, where uptake reflects the rate of phosphorylation, either adrenalectomy or hypophysectomy causes a substantial increase in uptake indicating removal of factors inhibiting the phosphorylation process (experiments 6 and 8). Further evidence for this inhibition is supplied in experiments 9 and 10. Here it is seen that pretreatment of the hypophysectomized-diabetic animal with growth hormone or cortisone partially restores the inhibition of phosphorylation. When both substances are administered together (experiment 11), phosphorylation is depressed below the level found in the simple diabetic (experiment 4). It can also be seen, in experiment 11, that the intracellular free glucose rises to a very high level, suggesting that the transport step is not grossly impaired.

In the above experiments the failure of insulin in vitro to relieve the depressed phosphorylation by diabetic muscle is not consistent with the fact that insulin in vivo completely restores to normal the peripheral utilization of glucose in the diabetic animal. In order to study this point further, experiments were undertaken to determine the effect of pretreatment with insulin in vivo on the subsequent phosphorylation by the isolated heart. It was found that treatment of the animal for one hour improves phosphorylation rates in some instances but not in others; treatment for four hours consistently restores the rate to normal and treatment for twenty-four hours leads to above normal rates (experiment 12). It is not clear whether this insulin effect is direct or indirect. If direct, it is slow, possibly because of slow penetration of the hormone through the membrane. If indirect, it does not appear to be due to suppression of pituitary and adrenal activity since the concomitant administration of growth hormone and cortisone does not modify the insulin response (experiment 13).

The depression of phosphorylation with diabetes and the restoration to normal with insulin in vivo are not due to adaptive changes in the enzyme content of the muscle. This is shown by the fact that exposure of the heart to anoxia immediately elevates the phosphorylation rate to a high level (experiment 14), and a subsequent re-exposure to aerobic conditions immediately returns phosphorylation to the usual diabetic rate. These rapid changes in activity demonstrate that we deal with inhibitory and not adaptive phenomena. It may be noted in passing that the sensitivity of phosphorylation to anoxia may be related to the lability of the inhibition of phosphorylation in muscle extracts mentioned earlier.

In the present discussion, it appears desirable to refer to effects on phosphorylation rather than to effects on the hexokinase reaction specifically. Studies of glycogen and glucose-6-phosphate levels in the heart, to be reported later, suggest that the primary site of pituitary and adrenal activity may be on a step beyond the hexokinase reaction. Inhibition of hexokinase may be secondary to the accumulation of a metabolic intermediate, possibly the known inhibitor glucose-6-phosphate.

As already mentioned, pituitary and adrenal secretions do not appear to inhibit the transport step. It should be emphasized, however, that these observations were made in tissues virtually free of insulin. Evidence has been presented earlier that pituitary and adrenal factors may affect transport rates in hearts from hypophysectomized animals where some insulin remains in the muscle.[22] Futhermore, it has been shown that the acceleration of transport with insulin is slow in diabetic muscle where pituitary and adrenal activity is probably greater than normal.[22] It seems therefore possible that pituitary and adrenal factors, while having no direct effect on transport, may nevertheless reduce the sensitivity of this step to insulin acceleration.

Kipnis[20] has recently carried out studies of transport and phosphorylation of the glucose analog, 2-deoxyglucose, in diabetic muscle using the isolated rat diaphragm as the test system. The conclusions he has reached are very similar to those presented here and make it seem likely that the general concept outlined below can be properly applied to muscle tissue in general.

SUMMARY

The role of glucose transport and phosphorylation in the uptake of glucose by diabetic muscle is discussed and illustrative data are presented. Transport of glucose

through the cell membrane is reduced due to a deficiency of insulin. This is the primary cause for the depressed uptake in the diabetic tissue since uptake is limited by the transport step. Insulin causes a marked increase in the rate of glucose transport. Glucose uptake does not increase to the normal extent, however, since phosphorylation is also depressed and becomes the rate-limiting step for uptake under these conditions. The depression of phosphorylation is due in part to adrenal and anterior pituitary activity, probably involving the growth hormone and hydrocortisone. Insulin relieves this depression but the hormone effect is delayed and may be indirect.

SUMMARIO IN INTERLINGUA

Transporto e Phosphorylation de Glucosa in le Musculo de Animales Diabetic

Le rolo del transporto e del phosphorylation de glucosa in le fixation de glucosa per musculo diabetic es discutite, e datos illustrative es presentate. Le transporto de glucosa a transverso le membrana cellular es reducite a causa de un deficientia de insulina. Isto es le causa primari del deprimite fixation de glucosa in le tissu diabetic, proque le fixation es limitate per le limitation del transporto. Le administration de insulina causa un marcate intensification del transporto de glucosa. Tamen, le fixation de glucosa non es augmentate usque al nivello normal, proque le phosphorylation es etiam deprimite e deveni sub iste conditiones le factor que restringe le fixation de glucosa. Le depression del phosphorylation es causate in parte per le activitate del adrenales e del pituitario anterior, in le qual le hormon de crescentia e hydrocortisona es probabilemente interessate. Insulina allevia iste depression, sed le effecto hormonal es retardate e es forsan indirecte.

ACKNOWLEDGMENT

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Plasma Insulin Concentrations in Nondiabetic and Early Diabetic Subjects

Determinations by a New Sensitive Immuno-assay Technic

Rosalyn S. Yalow, Ph.D., and Solomon A. Berson, M.D., New York City

For many years insulin was regarded as only weakly antigenic, or even, according to some, as essentially nonantigenic. However, a few years ago, during the course of investigations on the metabolism of I^{131}-labeled insulin, it was observed that the tagged insulin showed a prolonged retention in the blood stream of all subjects who had received insulin therapy, and it was demonstrated that this behavior was attributable to the presence of insulin-binding antibodies.[1]

Since the concentration of antibody is extremely low in most such patients and since the insulin-antibody complexes do not precipitate, certain special technics, including the use of an isotopically labeled insulin, are required for the detection of the antibody.[1] Further extension of these technics has made possible an immunologic method for the assay of very small amounts of insulin.

METHODS

When insulin is added to antiserum, the insulin and antibody molecules combine with each other to form insulin-antibody complexes, the rate of combination depending on the concentrations of the reactants and the activation energy of the reaction. Since the reaction is reversible,[1-4] the formed complexes continually undergo dissociation into free insulin and free antibody molecules. After a period of time, equilibrium is reached, i.e., the number of complexes being formed just equals the number of complexes undergoing dissociation, and the ratio of insulin bound in complexes to free insulin (denoted by B/F) remains constant thereafter. In mixtures in which the concentration of insulin is very low compared to the concentration of antibody molecules, there are always many uncomplexed antibody molecules readily available for reaction with the few insulin molecules and B/F is higher than in mixtures in which the insulin concentration is such that there is no marked excess of antibody molecules. Therefore, if a series of solutions is prepared, each with the same antibody concentration but with different insulin concentration (including a tracer quantity of insulin-I^{131}), a "standard curve" is obtained in which the B/F ratio decreases progressively with increase in insulin concentration although the total quantity of bound insulin increases gradually to an asymptotic level which represents complete saturation of the antibody (figure 1). By adding the same minute quantity of tracer insulin-I^{131} to unknown solutions which are incubated with antiserum in the same way, the insulin concentration present is determined by comparison of the observed B/F ratio with the standard curve (figure 2).

A brief description of the technics used in the determination of bound and free insulin should suffice here since these methods have been described fully elsewhere.[1,4] One hundred to 200 μL. of insulin-antiserum mixtures are applied to Whatmann 3 mm. paper strips and subjected to a combination of hydrodynamic flow chromatography and electrophoresis for about an hour, after which time insulin bound to antibody has migrated about two and one half to three inches toward the anode,[1] while free insulin, which adsorbs firmly to the paper,[1,5] has remained at the site of application ("origin"). After drying, the strips are assayed in an automatic strip counter and characteristically show two peaks of radioactivity, one at the origin representing free insulin, the other antibody-bound insulin (figure 3). The higher the insulin concentration the smaller is the peak of bound insulin relative to that of free insulin. The B/F ratio is determined by planimetric measurement of the areas beneath the two peaks.

Although insulin-binding antibodies in man are formed in response to treatment with commercial insulin preparations containing beef and pork insulins, these antibodies react also with insulin from other species[6,7] (e.g., sheep, horse and human insulins) and may be used to assay any of these species-specific insulins.[8] However, the reaction of human insulin with human anti-beef, pork insulin serum is not quite strong enough to serve as the basis for an assay of the very low concentrations

Presented at the Symposium on Changing Basic and Clinical Concepts of Diabetes Mellitus sponsored by The Clinical Society of the New York Diabetes Association, Inc., on Oct. 16, 1959.

From the Radioisotope Service, Veterans Administration Hospital, Bronx 68, New York.

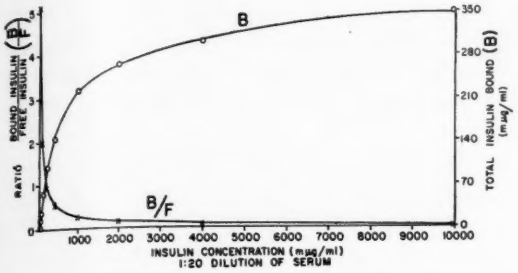

FIG. 1. Bound insulin (B) and ratio of bound to free insulin (B/F) as a function of beef insulin concentration in a human antiserum diluted 1:20.

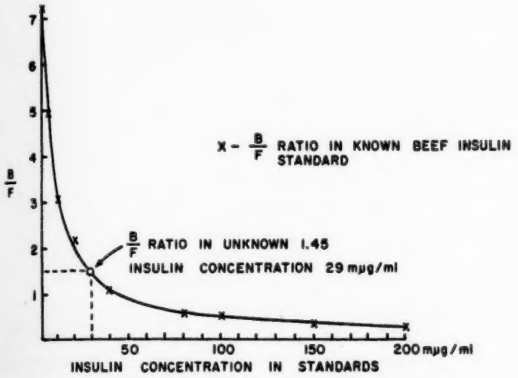
x - B/F RATIO IN KNOWN BEEF INSULIN STANDARD
B/F RATIO IN UNKNOWN 1.45
INSULIN CONCENTRATION 29 mμg/ml
B/F
INSULIN CONCENTRATION IN STANDARDS
200 mμg/ml

FIG. 2. Ratio of bound to free insulin (B/F) as a function of insulin concentration in known standards (x - x). The B/F ratio in an unknown sample (o) was 1.45 which corresponded to an insulin concentration of 29 mμg./ml.

present in plasma. Fortunately, antiserums from guinea pigs immunized with Protamine Zinc Insulin (beef) serve admirably for this purpose[8] and permit detection of a fraction of a microunit of human insulin.[9] Since a crystalline preparation of human insulin is not available, I^{131}-labeled beef insulin is utilized as the tracer although other crystalline labeled insulins might serve as well. The strongly competitive cross-reaction of human insulin makes it possible to obtain standard curves for the B/F ratio of beef insulin-I^{131} as a function of the concentration of human insulin at very low human insulin concentrations (figure 4).

Since, as might be expected, beef insulin acts somewhat more strongly with the antibody than does human insulin (figure 4), it is essential to keep the beef insulin-I^{131} concentration as low as possible and at the same concentration in both standards and unknowns. A standard curve for human insulin should be run with each lot of insulin-I^{131}, although, when the same amount of beef insulin-I^{131} is employed in different runs with the

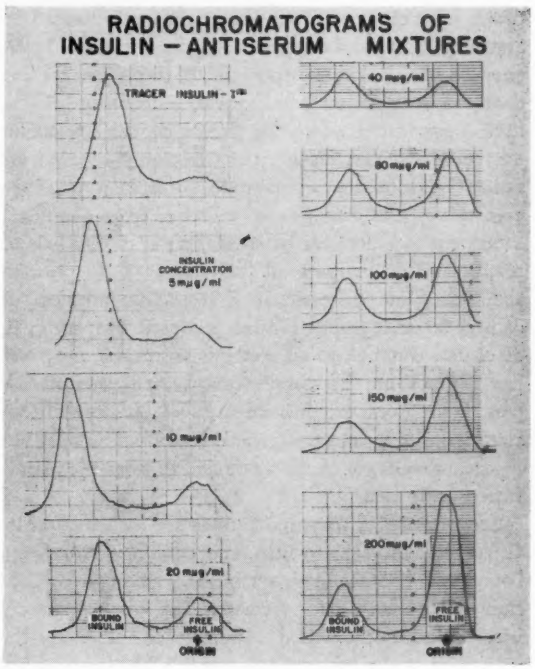

FIG. 3. Radiochromatograms which provided the data for the known standards plotted in figure 2.

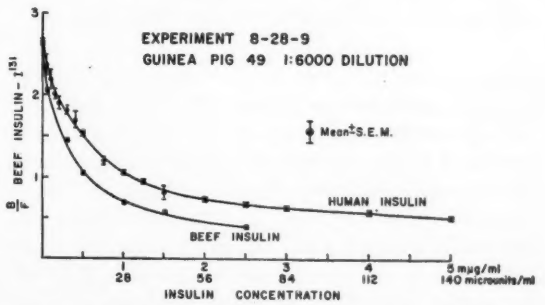

FIG. 4. Ratio of bound to free beef insulin-I^{131} as a function of human insulin concentration (● - ●) and beef insulin concentrations (■ - ■) in known standards.

same dilution of antiserum, the standard curves are quite reproducible.

Several technical details are to be observed. First, the available commercial preparations of labeled insulin do not have a sufficiently high specific activity and are, therefore, unsuitable for the present purpose. The insulin-I^{131} preparations used in this study had specific activities averaging about 100 mc. I^{131}/mg. insulin. At this level, damage to the insulin (by irradiation and other factors) is a problem.[10,11] With appropriate measures it has been possible to limit the extent of damage to be-

tween 3 per cent and 6 per cent of the total insulin-I^{131}. Since the damaged components migrate with serum proteins on chromato-electrophoresis, a correction for the damaged fraction must be applied. Moreover, plasma itself alters insulin and, aside from a degradative action on the endogenous insulin, the effect on the added insulin-I^{131} is somewhat similar to that of irradiation. However, this type of damage can be limited to an additional 1 per cent to 3 per cent by employing 1:10 dilutions of plasma and maintaining all mixtures at 4° C. prior to and during chromato-electrophoresis. The problem of damage to endogenous insulin produced by plasma is, of course, common to all methods of insulin assay and has perhaps not been sufficiently emphasized in the past. It has been the practice in this laboratory to separate the plasma in a refrigerated centrifuge immediately after withdrawal of the blood and to keep the plasma frozen until used.

Standard and unknown solutions are incubated at 4° C. for four days by which time equilibrium between bound and free insulin is established, although equilibrium is not essential for the assay. The low temperature incubation has the added advantage of increasing the sensitivity of the test, a finding which had been anticipated from a thermodynamic study of the insulin-antibody reaction.[4] A more detailed description of the methods employed here has been prepared for publication.[12]

RESULTS

Experiments on the recovery of human insulin added to plasma show that the plasma itself has no inhibitory or augmentive effects (table 1) and this conclusion is confirmed by the proportional decrease in measured insulin concentration observed on dilution of the plasma (figure 5). It may be noted that the reaction is not only specific for insulin but also that different species-specific insulins show as much as ten- to a hundredfold differences in their affinity for specific insulin antibodies.[5]

TABLE 1

Recovery of human insulin added to 1:10 dilution of fasting specimen of Ku.

Human insulin added	Observed human insulin concentration	Per cent of added insulin recovered
0.0 mμg./ml.	0.17 mμg./ml.	
0.5	0.62	90
	0.64	94
0.75	0.92	100
	0.92	100
1.0	1.28	111
	1.18	101
1.5	1.75	105
	2.08	127

The results presented here include (1) plasma insulin concentrations in nondiabetic subjects under fasting conditions and during a standard two-hour 100 gm. oral glucose tolerance test, and (2) plasma insulin concentrations, under similar conditions, in subjects with newly discovered maturity-onset diabetes, exclusive of those who had ever been treated with insulin. Fourteen and seventeen subjects in each group, respectively, have been studied. With one exception, the criteria for diagnosis were based solely on the blood sugar curve during the glucose tolerance test (figure 6). All diabetic subjects showed a peak blood sugar concentration over 180 mg. per 100 ml. or two consecutive blood sugar concentrations exceeding 160 mg./100 ml. In all but one of the nondiabetic subjects, the peak blood sugar concentration did not exceed 160 mg./100 ml. The single exceptional patient (Al) had a marginal ulcer and dumping syndrome which was considered an adequate explanation for the high blood sugar concentration at one-half hour and the precipitate decline thereafter.

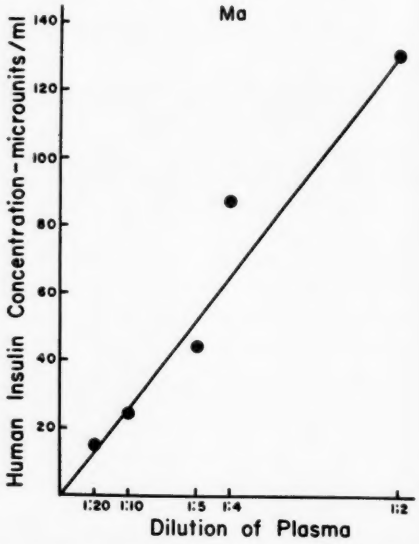

FIG. 5. Human insulin concentration as a function of plasma dilution in a specimen taken one-half hour after oral glucose administration. This subject is not included in the series of patients presented in figure 7.

Mean fasting insulin concentrations in diabetic (29 μU./ml.) and nondiabetic (24 μU./ml.) subjects did not differ significantly (figure 7), the highest concentration in either group not exceeding 66 μU./ml. However, it should be emphasized that fasting blood sugar concentrations were significantly elevated in only a few members of this group of diabetic subjects. The insulin

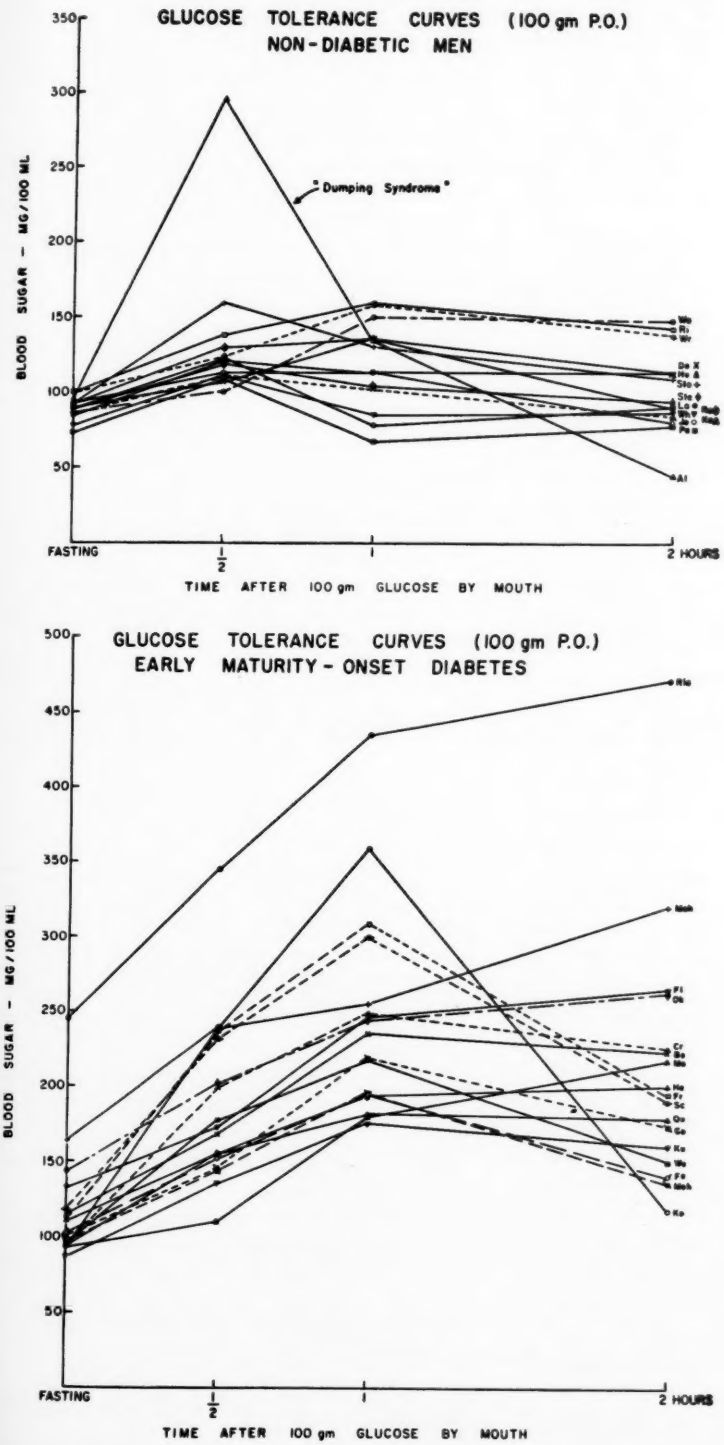

FIG. 6. Blood sugar curves in nondiabetic (top) and early maturity-onset diabetic (bottom) subjects during a standard two-hour glucose tolerance test (100 gm. p.o.).

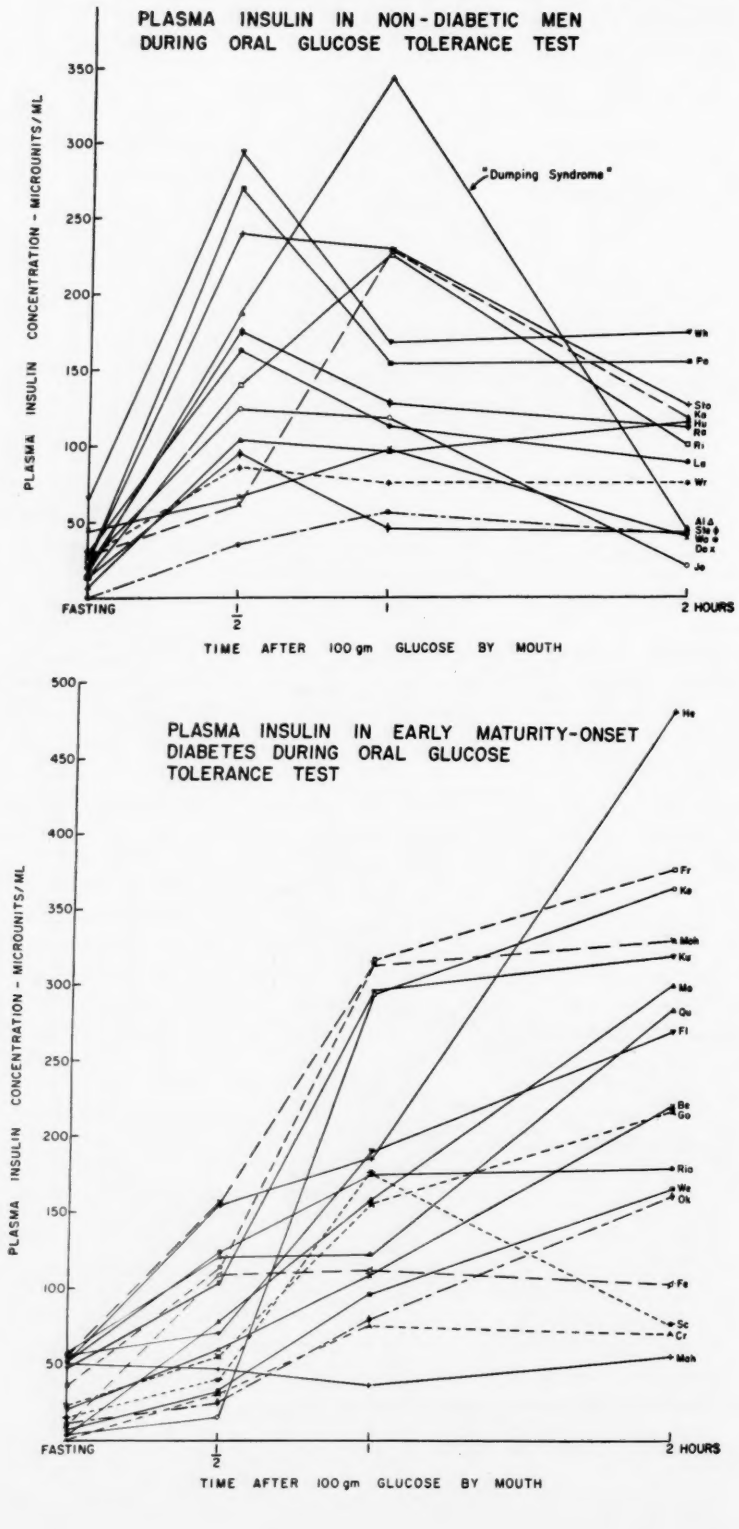

FIG. 7. Plasma insulin concentrations in nondiabetic (top) and early maturity-onset diabetic (bottom) subjects during a standard two-hour glucose tolerance test. The blood sugar curves for these subjects are given in figure 6.

concentration curves following glucose administration showed striking differences between the two groups of subjects (figure 7). The nondiabetic subjects were equally divided in showing their peak insulin concentrations at one-half hour or at one hour. In contrast, the diabetic patients at one-half hour generally showed a lesser increase in insulin concentration above the fasting level but their insulin concentrations continued to increase at one hour and in almost all diabetic patients the concentrations were highest at two hours. There were wide variations in individual insulin concentration curves in both groups. The mean curves for each group reveal the different patterns of insulin secretory response to glucose administration (figure 8).

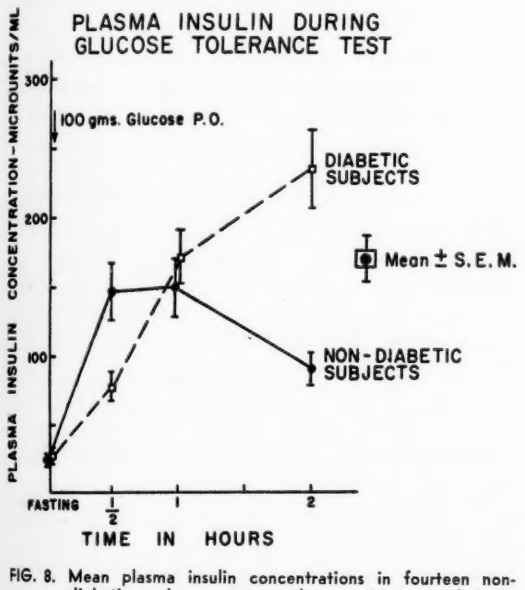

FIG. 8. Mean plasma insulin concentrations in fourteen nondiabetic and seventeen early maturity-onset diabetic subjects during a standard two-hour glucose tolerance test (100 gm. p.o.). The individual values for these subjects are given in figure 7.

In addition to the effects of glucose on the plasma insulin concentration the effects of sodium tolbutamide 1 gm. intravenously in twelve patients and 3 gm. orally in ten patients have been studied and are to be reported in detail elsewhere.[28] An increase in insulin concentration in peripheral blood occurred in every case, but the increase was almost always much less marked than that produced by glucose.

DISCUSSION

The method of insulin assay described here employs 0.2 ml. of mixtures containing 20 μL. of plasma and the standard curve is routinely obtained over the concentration range of 1.4 to 140 μU. per ml. It is evident (figure 4) that there is a definite fall in the B/F ratio even at the lowest concentrations of 1.4 to 2.8 μU. per ml. so that the absolute amount of insulin detectable was two tenths of these amounts or considerably less than .1 μU. In other experiments employing a still greater dilution of the same antiserum, 0.1 μU. of insulin was detectable. To our knowledge no other insulin assay approximates these sensitivities.

An added advantage of the immuno-assay method is the absence of nonspecific effects of plasma itself as shown by the plasma dilution and insulin recovery experiments. By combining the immunologic assay with the diaphragm or fat pad assays for "insulin-like activity" it may be possible to investigate, more fully than heretofore possible, the plasma inhibitors and noninsulin stimulators of glucose metabolism.

In light of the present studies there appears little doubt that insulin secretion is at least delayed in the early mature diabetic. This delay may be due either to a higher glucose concentration threshold for stimulation of insulin secretion or to an inability to produce or secrete insulin at a sufficiently rapid rate. Since these patients were not followed past the two-hour point, it is not known whether the insulin concentration continues to rise thereafter in the diabetic patients. Although the average insulin concentration at two hours in the diabetic group exceeds the highest average concentration in nondiabetic patients, it cannot be concluded that the early diabetic is capable of putting out as much insulin as the nondiabetic, since the latter may well have exhibited very much higher values in the presence of the same degree of prolonged hyperglycemia as existed in the diabetic subjects. This possibility has yet to be tested. However, in support of this suggestion are the observations in Al, a nondiabetic subject whose marginal ulcer and dumping syndrome were responsible for very rapid glucose absorption and a blood sugar concentration of 296 mg./per 100 ml. at one-half hour (figure 6). His plasma insulin concentration at one hour was the highest observed at any time among the nondiabetic subjects (figure 7) and his blood sugar fell precipitously to 44 mg./per 100 ml. at two hours.

Finally, the apparent difference in blood sugar response to endogenous insulin must be commented upon. Before rational postulates can be made regarding the pathogenesis of diabetes, it must be questioned how a very high average blood sugar concentration can be resolved with a higher than normal integrated insulin output in diabetic subjects during the glucose tolerance test. Even when cognizant of the delay in insulin secre-

tion, it is puzzling that the diabetic should continue to show hyperglycemia after one hour when his insulin concentration at that time is as high as in the nondiabetic. Furthermore, in individual cases, the insulin concentration at one-half hour in some diabetics exceeds that of some nondiabetics. Yet the blood sugar concentration falls in the latter and continues to rise in the former patients. Recently Baird and Bornstein,[14] employing adrenalectomized diabetic mice for insulin assay, have reported higher than normal insulin concentrations in two diabetic subjects, although normal levels in two patients and no detectable insulin concentrations in two others were also encountered. It was not stated whether these were fasting or postprandial blood specimens. However, these results are in accord with those of the present study in suggesting that the tissues of the maturity-onset diabetic patient do not respond to his insulin as well as do the tissues of the nondiabetic to his insulin. Whether this phenomena is due to abnormal tissues, to an abnormal insulin, or to the presence of insulin antagonists, remains for future investigations to answer.

SUMMARY AND CONCLUSIONS

A specific, precise and sensitive assay for insulin in human plasma has been described. The method depends on the competitive inhibition by microunit quantities of human insulin of the binding of beef insulin-I^{131} to insulin-binding antibodies in the sera of guinea pigs immunized with beef insulin. Application of this assay method to preliminary studies on endogenous plasma insulin concentrations during the glucose tolerance test in maturity-onset diabetic patients and nondiabetic subjects is presented. The insulin concentration curves during the glucose tolerance test showed characteristic differences between the two groups of patients. Whereas fasting insulin concentrations did not differ significantly between diabetic and nondiabetic subjects, the mean insulin secretory response to glucose was delayed in diabetic subjects but eventually exceeded that observed in the nondiabetic group.

SUMMARIO IN INTERLINGUA

Concentrationes de Insulina in le Plasma de Subjectos Non-Diabetic e Precocemente Diabetic: Determinationes per Medio de Un Nove Technica Sensibile de Immuno-Essayage

Es describite un methodo specific, precise, e sensibile pro le determination de insulina in plasma human. Le methodo depende del inhibition competitive, per quantitates in microunitates de insulina human, del ligation de radio-iodate insulina bovin a anticorpore insulino-ligatori in le sero de porcos de India immunisate con insulina bovin. Le application de iste methodologia a studios preliminari del concentrationes de endogene insulina plasmatic durante le test del tolerantia pro glucosa in patientes con diabete a declaration a etates matur e in subjectos non-diabetic es presentate. Le curvas del concentration de insulina durante le test de tolerantia pro glucosa exhibiva differentias characteristic inter le duo gruppos de patientes. Durante que le concentrationes de insulina in stato jejun non differeva significativemente inter le subjectos diabetic e le subjectos non-diabetic, le responsa medie de secretion de insulina evocate per glucosa esseva retardate in subjectos diabetic, sed in le curso del tempore illo excedeva le correspondente effecto observate in le gruppo non-diabetic.

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Metabolic Role of Glucose

A Source of Glyceride-glycerol in Controlling the Release of Fatty Acids by Adipose Tissue

F. C. Wood, Jr., M.D., B. Leboeuf, M.D., and G. F. Cahill, Jr., M.D., Boston

Current knowledge suggests that the absence of effective insulin, as in uncontrolled diabetics, results in decreased transport of glucose across the cell wall in certain tissues. This defect, in conjunction with accelerated hepatic gluconeogenesis, produces hyperglycemia and overflow glycosuria. In addition, there is concomitant lipid mobilization as evidenced by ketosis and hyperlipemia. Recently attention has been drawn to albumin-bound plasma fatty acids as a major form of lipid transport from adipose tissue stores to sites of utilization.[1,2] A rise in the level of these fatty acids is currently the first detectable evidence of increased lipid mobilization, and it is therefore apparent that a better understanding of the mechanism of fatty acid release from adipose tissue might partially clarify the genesis of diabetic ketoacidosis.

Several investigators[3-5] have demonstrated a decreased plasma fatty acid concentration in man following administration of insulin or glucose. Conversely, plasma fatty acids are increased in patients with uncontrolled diabetes. Bierman, Schwartz and Dole[6] and Gordon[5] have concluded that the major insulin effect on the regulation of plasma fatty acid concentration is at the site of release from adipose tissue and not at the point of uptake or utilization by tissues such as muscle.

Recent studies from this laboratory[7-11] indicate that the effects of glucose and insulin on fatty acid mobilization are mediated at least in part through their action upon esterification of fatty acids in adipose tissue. This conclusion was first suggested by observations concerning the comparative incorporation of labeled glucose carbon and glycerol carbon into the glycerol of adipose tissue lipids (glyceride-glycerol). In rat epididymal adipose tissue, glucose carbon is incorporated into glyceride-glycerol at a rate many times greater than carbon from glycerol itself,[11] and in vivo studies have corroborated this rapid incorporation of glucose carbon into glyceride-glycerol.[11]

A recent abstract[12] suggests that adipose tissue homogenates require α-glycerophosphate or one of its phosphorylated precursors for optimal esterification of palmitate-1-C^{14} whereas glycerol itself is ineffective. This finding corroborates similar reports for liver[13] and intestinal mucosa.[14] If the esterification process in intact adipose tissue also requires α-glycerophosphate, and if glycerol itself is esterified slowly or not at all in this tissue, then esterification should be limited by the rate of formation of α-glycerophosphate from glucose via dihydroxy-acetone phosphate.

It is known that adipose tissue triglycerides are constantly undergoing hydrolysis and re-esterification within the tissue.[11] In the steady state, when there is neither net synthesis nor net release of fatty acids, it is reasonable to assume that fatty acids released from triglyceride are re-esterified as fast as they are formed. The glycerol produced is free glycerol[15] and thus essentially unavailable for re-esterification. It is therefore necessary in this situation to assume a continuous supply of phosphorylated glycerol, presumably from glucose. If these assumptions are correct, then it is likely that decreased availability of glucose as a source of glyceride-glycerol (as in fasting or diabetes) would impair fatty acid re-esterification with consequent increase in tissue-unesterified fatty acids from continuing triglyceride hydrolysis. As a result plasma free fatty acids should increase. On the other hand, if the source of glyceride-glycerol is increased (as following glucose or insulin) fatty acid levels should decrease as augmented esterification diminishes the tissue free fatty

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From the Departments of Medicine, Harvard Medical School and the Peter Bent Brigham Hospital, and the Baker Clinic Research Laboratory, New England Deaconess Hospital, Boston, Massachusetts.

acid pool. Thus, the balance between triglyceride hydrolysis and re-esterification may be partially controlled by the direct influence of available glucose on the re-esterification process.

To test the above hypothesis the incorporation of glucose carbon into glyceride-glycerol has been measured under conditions of varying availability of glucose. Rat epididymal adipose tissue was incubated with glucose randomly labeled with C^{14} in Krebs-Ringer bicarbonate buffer. The incorporation of glucose carbon into CO_2, glycogen, fatty acids and glyceride-glycerol was measured. Figure 1 summarizes the total metabolism of glucose to recovered products (total bars) and specifically to glyceride-glycerol (black portion of the bars). The metabolic states represented are, from left to right: (a) tissue from fed rats incubated with insulin, (b) tissue from fed rats incubated without insulin, (c) tissue from rats fasted twenty-four and seventy-two hours, and (d) tissue from alloxan-diabetic rats. These represent metabolic states of decreasing glucose metabolism and it is not surprising, therefore, that the absolute amount of glyceride-glycerol formed from glucose progressively decreases. It is of interest, however, that, as less glucose is metabolized, a greater *percentage* of glucose carbon is recovered in glyceride-glycerol. Thus, in the diabetic animal, more than 80 per cent of metabolized glucose carbon is utilized for glyceride-glycerol synthesis. Since the metabolic states, as shown in the figure, are also characterized by an increasing rate of release of free fatty acids from adipose tissue, it would appear that an inverse correlation exists between total glyceride-glycerol synthesis and the release of free fatty acids. The increasing percentage of glucose metabolized to glyceride-glycerol can be interpreted as the tissue's attempt to moderate by re-esterification the rate of free fatty acid release.

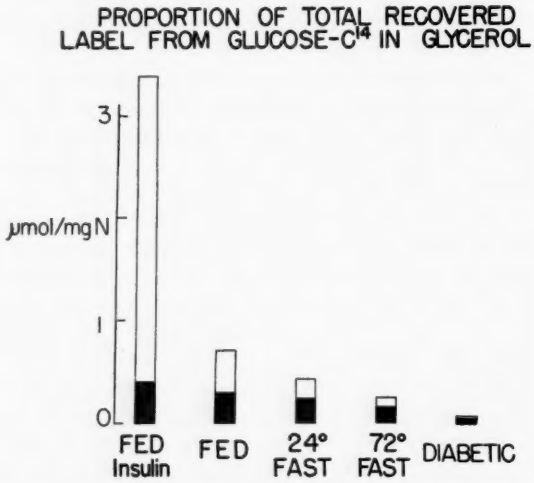

FIGURE 1

SUMMARY

These observations support the concept that, under certain conditions, the carbohydrate action upon the release of free fatty acids is mediated in large part through the ability of the carbohydrate to act as an effective metabolic precursor for glyceride-glycerol. This mechanism is likely to be important in overfeeding or starvation, and in the metabolic response to excess or lack of insulin. It is, however, probably not the only mechanism regulating the release of fatty acids from adipose tissue. For example epinephrine, norepinephrine, and ACTH and growth hormone preparations, which have been shown by many laboratories to increase fatty acid mobilization, appear to do so by increasing triglyceride breakdown rather than limiting re-esterification.[9,10,16]

These studies emphasize the importance of glyceride-glycerol formation in the metabolism of glucose by adipose tissue. Thus glucose in the presence of insulin may affect lipid storage not only as a source of fatty acid carbon and hydrogen, but also as a source of activated glycerol capable of esterification with fatty acids. Indeed, in certain instances, this last metabolic action of glucose may fulfill important regulatory functions.

SUMMARIO IN INTERLINGUA

Le Rolo Metabolic de Glucosa Como Fonte de Glyceride-Glycerol in le Regulation de Acidos Grasse per Tissus Adipose

Le hic-presentate observationes supporta le these que sub certe conditiones le effecto de hydrato de carbon super le liberation de libere acidos grasse es mediate in grande parte per le capacitate de hydrato de carbon de ager como un efficace precursor metabolic de glycerido-glycerol. Il es probabile que le mechanismo es importante in alimentation excessive e in affamation e in le responsa metabolic a excessos o mancos de insulina. Tamen, il es probabile que iste mechanismo non es le sol que es responsabile pro le liberation de acidos grasse ab le tissus adipose. Per exemplo, epinephrina, norepinephrina, e ACTH e preparatos de hormon de crescentia—que in multe investigationes laboratorial se ha monstrate capace de augmentar le mobilisation de acido grasse—pare producer iste effecto per augmentar le decomposition de triglycerido plus tosto que per restringer le re-esterification.[9,10,16]

Iste studios signala le importantia del formation de glycerido-glycerol in le metabolismo de glucosa per tissu adipose. Assi glucosa in le presentia de insulina affice le thesaurisation lipidic possibilemente non solo como fonte de carbon e hydrogeno de acido grasse sed etiam como fonte de activate glycerol capace de esterification con acidos grasse. De facto, in certe casos iste ultime action metabolic de glycosa executa possibilemente importante functiones regulatori.

ACKNOWLEDGMENT

This study was supported by grants from the U.S. Public Health Service, H-4569, Bethesda, Maryland, the Lipotropic Research Foundation, New York, New York, and Eli Lilly and Company, Indianapolis, Indiana.

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Metabolism and Obesity

Studies of experimental obesities have indicated that these can be divided into two general classes (J. Mayer, *Nutrition Abstr. and Rev.* 25:597, 1955). The "regulatory" obesities are the forms in which there is an abnormality of the central mechanism regulating food intake so that the energy intake of the animal is greater than its expenditure. This in turn may cause a number of metabolic changes secondary to the hyperphagia. Examples of such "regulatory" obesities are the surgically induced hypothalamic obesities in the cat, rat, mouse, etc., and goldthioglucose induced hypothalamic obesity in the mouse. By contrast, "metabolic" obesities are incurred when an abnormality in the metabolism of non-nervous tissue causes the animal to become obese as a secondary consequence of the metabolic abnormality. Examples of this second class are mice with the hereditary obese-hyperglycemic syndrome, mice bearing ACTH-secreting tumors, etc.

In man, such a typing of obesity has not been attempted systematically yet. Even the studies of metabolic abnormalities, whether primary or secondary to the obesity, have hardly been attempted.

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Effect of Insulinogenic Agents on the Pancreatic Islets

Bruno W. Volk, M.D., and Sydney S. Lazarus, M.D., M.Sc. (Med.), New York City

During the years following the histologic characterization of the pancreatic β cells and their identification as the source of insulin certain characteristic morphologic changes of this cellular system have been shown to occur under the stimulus of various diabetogenic hormones. These changes which may differ according to species and degree of diabetes include β cell degranulation, enlargement of nuclei and Golgi apparatus, increased mitotic activity of β cells, vacuolization of β cells and duct epithelium and, in some species, β cell destruction.

Since glucose administration[1-7] as well as partial pancreatectomy[8,9] or hormonal treatment[10-25] cause similar changes in pancreatic morphology, it has been postulated that hyperglycemia is the common pathogenetic agent.[1,2,7,18] Furthermore, since all these morphologic changes occur sequentially they have been lumped together as resulting from functional stimulation of β cells.[18-20] A logical extension of this idea was the suggestion that the destruction of β cells in dogs and cats during experimental diabetes is a result of functional exhaustion of these cells.[2,12,20,21]

More recently it has been shown that the chronic administration of various sulfa drugs causes extensive or complete degranulation of β cells.[22-26] This degranulation is the result of increased insulin output due to a direct action of the drug on this cellular system.[26-27] However, in contrast to the effect of hyperglycemia, no other pancreatic alterations are seen.

The present paper constitutes an attempt to demonstrate the functional significance of the various structural alterations of the pancreatic islets and their relationship to the prolonged administration of various stimulating agents.

Presented at the Symposium on Changing Basic and Clinical Concepts of Diabetes Mellitus sponsored by The Clinical Society of the New York Diabetes Association, Inc., on Oct. 16, 1959.

From the Isaac Albert Research Institute of the Jewish Chronic Disease Hospital, Brooklyn, and the Department of Pathology, Albert Einstein College of Medicine, Bronx, New York.

EXPERIMENTAL EFFECT OF CHRONIC HYPERGLYCEMIA ON PANCREATIC MORPHOLOGY

1. *Rabbits.* To produce chronic hyperglycemia in the rabbit, animals were treated with (a) 4 mg./kg. daily of cortisone* for twenty-one days and 8 mg./kg. thereafter; (b) 1 mg./kg. of cortisone acetate daily together with 1 gm. of crystalline glucagon† in oil three times a day at eight-hour intervals; (c) 1 mg./kg. of cortisone acetate daily and 2 mg. adrenalin in oil three times a day at eight-hour intervals. Surviving animals were sacrificed at intervals of from one to 106 days.

Figure 1 demonstrates typical blood sugar responses for each of these three regimens. It can be seen that moderate or severe diabetes developed in each instance but that the hyperglycemic plateau was not maintained.

The sequence of morphologic alterations in the pancreas that was observed during each type of treatment was similar. Beta cell degranulation appeared within two days. Concomitantly, with increasing blood sugar levels this eventually became very marked or even complete (compare Figures 2a and b). The β cells became larger than normal as did their nuclei, which became more ovoid with more sparsely distributed chromatin than in the normal β cell. Increased mitotic activity of the β cells was noted between the third and tenth day. Glycogen vacuolization occurred in ductular epithelium usually within five to six days and then also appeared in the β cell. Its extent was related to the severity and duration of the diabetes. In those animals in which hyperglycemia was maintained over prolonged periods of time, most ductular epithelial cells were vacuolated. However, additional nonvacuolated hyperplastic ductules were found within or contiguous with

*Cortisone acetate was supplied by The Upjohn Company, Kalamazoo, Michigan, through the courtesy of Dr. C. J. O'Donovan.

†Crystalline glucagon (Lot #258-234B-54-2) was supplied by the Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana, through the courtesy of Dr. W. R. Kirtley.

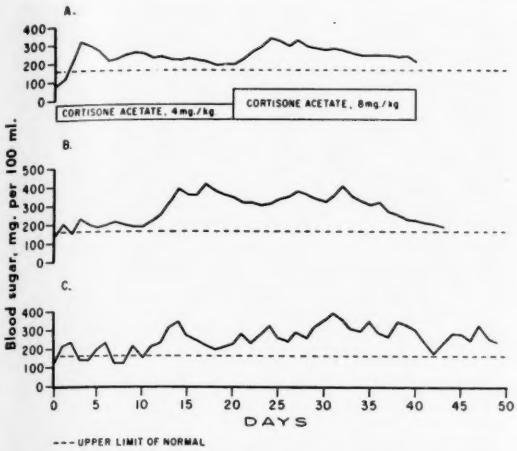


FIG. 1. Morning blood sugar levels of three representative rabbits: (A) treated with 4 mg./kg. followed by 8 mg./kg. of cortisone-acetate; (B) daily mean blood sugar levels of a rabbit treated with 1 mg. of crystalline glucagon in oil subcutaneously three times a day and 1 mg./kg. of cortisone-acetate intramuscularly daily; and (C) mean blood sugar level of a rabbit treated with 2 mg./kg. of adrenalin in oil subcutaneously three times a day and 1 mg./kg. of cortisone-acetate once daily.

islets. In occasional instances primitive β cells were seen originating from this type of hyperplastic ductular epithelium (figure 3). These cells showed large nuclei with abundant opaque somewhat reticulated cytoplasm. Also, there were seen within islets young β cells with sparse, diffuse aldehyde fuchsin positive granules or at times abundant granules. These cells were distinguished from adjacent older β cells by the fact that the latter were smaller, degranulated and showed aldehyde fuchsin positive cell borders. In the animals treated for the

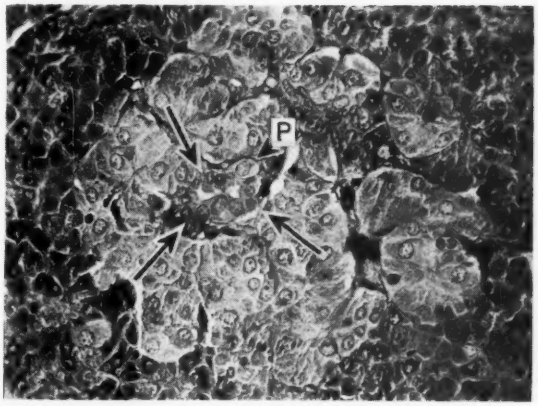
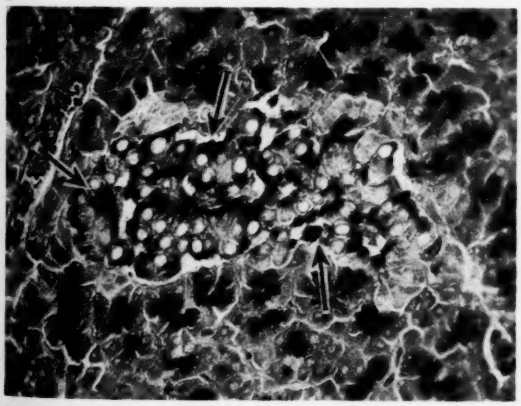


FIG. 3. Pancreas of a rabbit treated for 106 days with 1 mg. of crystalline glucagon three times daily together with 1 mg./kg. of cortisone-acetate daily. The islet is enlarged and irregular and shows β cell degranulation. A hyperplastic duct (arrows) containing a primitive β cell (P) is present. Aldehyde Fuchsin Trichrome. X 210.

longest periods of time, the islets were markedly hyperplastic with irregular contours and visual estimates of their volume showed them to be definitely increased over those seen in normal rabbits.

2. *Dogs.* Chronic hyperglycemia was produced in the dog by administration to 50 per cent partially pancreatectomized animals of 3 mg./kg. of growth hormone* subcutaneously daily for up to twenty-one days. The animals showed a rise in blood sugar level within forty-eight hours. The hyperglycemia increased progressively and reached a plateau, which persisted even

*Purified growth hormone was a gift from the Endocrine Study Section of the National Institutes of Health.



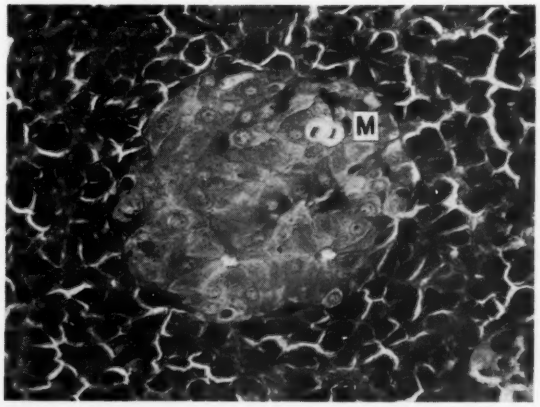


FIG. 2. (a at left) Pancreatic islet of untreated rabbit showing α and β cells. The β cell granules are polarized at the capillary borders of the cells (arrows). Aldehyde Fuchsin Trichrome. X 297. (b at right) Islet of rabbit treated for five days with 4 mg./kg. of cortisone-acetate. There is extensive β cell degranulation with occasional residual granules and a mitotic figure (M). Alpha cells are black. Aldehyde Fuchsin Trichrome. X 297.

when hormonal treatment was discontinued after twenty-one days (figure 4A). Morphologically, β cell degranulation became pronounced after forty-eight hours. At this time there was also increased glycogenization of ductular epithelium. Beta cell glycogenization first became apparent at four days and gradually became more extensive. At about nine or ten days, in animals with extensively vacuolated ductular epithelium, fat as well as glycogen could be demonstrated in the vacuoles.

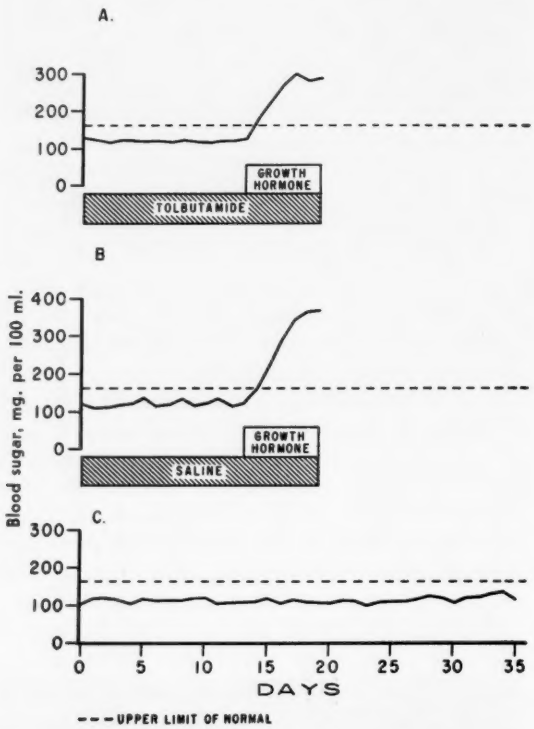


FIG. 4. Daily morning blood sugar levels of three representative 50 per cent partially pancreatectomized dogs which received respectively (A) 0.5 gm. twice daily orally of tolbutamide and later also 3 mg./kg. of crystalline growth hormone subcutaneously; (B) saline solution and later also 3 mg./kg. of growth hormone daily subcutaneously; (C) only 0.5 gm. twice daily orally of tolbutamide.

Within a few days after starting growth hormone an additional alteration was seen in scattered individual islet cells. These cells were ballooned out and showed reticulated vacuolated cytoplasm containing neither glycogen nor fat (figure 6). The nuclei showed gradations of pyknosis suggestive of early loss of cellular viability (figure 7). After fourteen to twenty-one days of treatment with growth hormone, islet tissue was difficult to

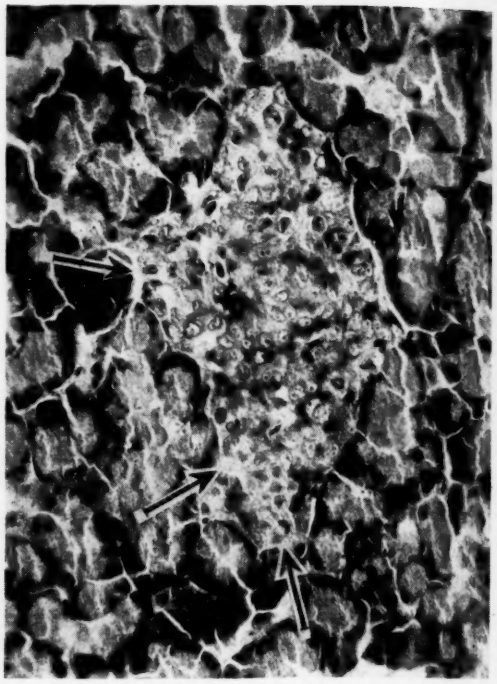


FIG. 5. Pancreatic islet of normal dog showing well granulated β cells. The α cells are paler (arrows). Aldehyde Fuchsin Trichrome. X 265.

find in the pancreas. Occasional residual islets were composed mostly of α cells and agranular cells of unidentifiable antecedents. No evidence was found of neogenesis of β cells either from ductular epithelium from acinar tissue or from other cell types in the dog pancreas. Occasional mitoses were found in β cells, however. Equal numbers of mitoses were also found in duct epithelium as well as in acinar cells.

EFFECT OF HYPOGLYCEMIC SULFONYLUREAS ON PANCREATIC MORPHOLOGY IN RABBITS AND DOGS

Rabbits received into the marginal ear veins 0.5 mg. of tolbutamide, metahexamide or chlorpropamide and were sacrificed at intervals of from one to thirty-five days. Dogs received 0.5 gm. orally of tolbutamide twice a day for up to eighty-three days. The morning blood sugar values of treated and untreated animals of either species were within normal limits. Morphologically, no changes were seen in either the α or δ cells or in the exocrine portion of the pancreas. The β cells showed varying degrees of degranulation which intensified as the duration of therapy was prolonged. The nuclei

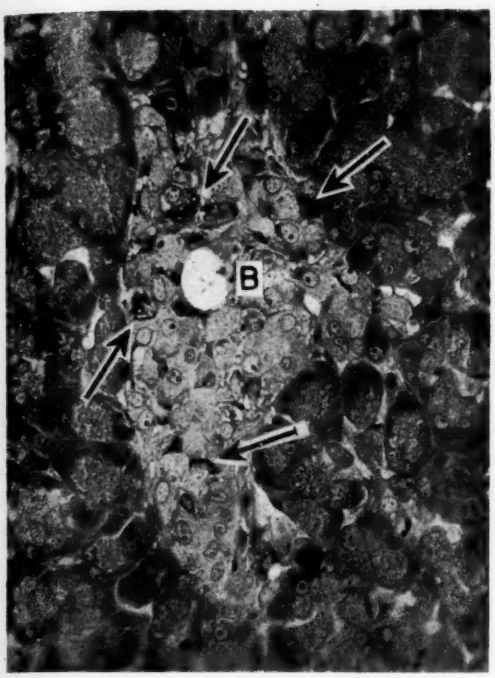


FIG. 6. Pancreas of 50 per cent partially pancreatectomized dog treated with 0.5 gm. of tolbutamide twice daily orally and later also with 3 mg./kg. of growth hormone subcutaneously. There is glycogenization of β cells (arrows) as well as a ballooned-out necrobiotic, nonglycogen containing β cell (B). PAS-Trichrome. X 265.

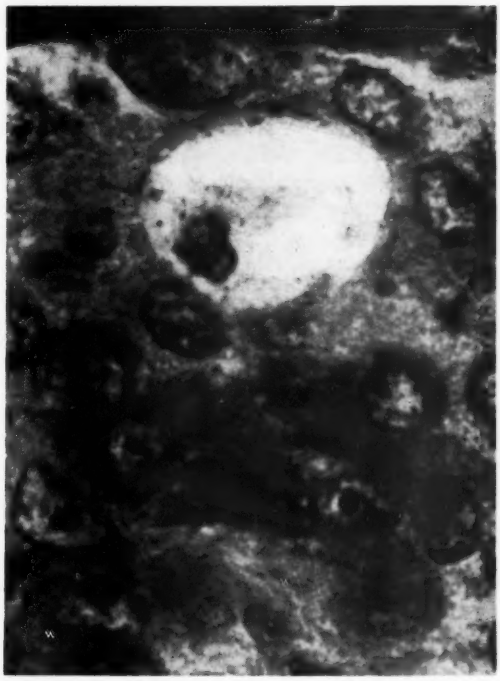


FIG. 7. Islet of 50 per cent partially pancreatectomized dog treated with 0.5 gm. of tolbutamide twice daily orally and later also with 3 mg./kg. of growth hormone. There is a ballooned-out vacuolated β cell with pyknotic nucleus seen. PAS-Trichrome. X 1,920.

generally became considerably enlarged and the chromatin was sparsely distributed, being concentrated around the nuclear membranes. The negative image of the Golgi apparatus also appeared to be enlarged (see figure 8). In no instance was any increased mitotic activity of β cells noted nor was there any evidence of new β cell formation from duct epithelium, acinar tissue or any other cell types. Furthermore, no proliferation of ductules was observed. This was so in both the rabbit and the dog even after prolonged continuous treatment with various sulfonylurea drugs.

EFFECT OF DIABETOGENIC HORMONES ON PANCREATIC MORPHOLOGY IN SULFONYLUREA TREATED RABBITS AND DOGS

In order to determine the effect of two separate stimuli on the pancreas, rabbits were pretreated with 125 mg./kg. of metahexamide intravenously daily for fourteen days and then received in addition 4 mg./kg. of cortisone for up to nineteen days. Also 50 per cent partially pancreatectomized dogs were treated with 0.5 gm. of tolbutamide orally for fourteen days and then also received 3 mg./kg. of purified growth hormone subcutaneously for up to eight days. In the rabbit the degree of diabetes which developed was similar to that which had been previously observed with cortisone alone. Morphologically, the pancreas showed changes which correlated with the degree of diabetes and included degranulation and glycogen infiltration. In the dog, despite concomitant sulfonylurea administration, a marked hyperglycemia similar to that which had appeared in the animals which received growth hormone alone developed within the second to sixth day after the beginning of hormonal treatment (figure 4b). The diabetes continued until sacrifice or spontaneous death of the animal. Morphologically, the changes in the β cells were similar to those in animals treated with growth hormone alone, except that degranulation of β cells was much more extensive at an earlier stage.

DISCUSSION

Beta cell granules are thought to represent a precursor of insulin and correlate with the insulin content

of the pancreas.[28–31] It has been assumed that β cell degranulation could represent either an increased rate of liberation of insulin from β cells or a diminished rate of formation of insulin by these cells. Experimentally, it has been shown that fasting or a high fat diet or the administration of exogenous insulin decreases β cell granulation and also the insulin content of the pancreas.[26,29,33–34] In these instances it is assumed that the β cell degranulation indicates suppression of insulin formation. On the other hand, glucose administration has been shown to degranulate the pancreas,[3–6] diminish pancreatic insulin content[26] and, at the same time, increase the output of insulin from the pancreas.[7] This latter has been confirmed, in vitro, by perfusion of rat pancreas by hyperglycemic blood and assay of the perfusate for its insulin content.[35] Therefore, in the pancreas degranulated by hyperglycemia, although the β cells are producing insulin more readily than in the normal, the rate of liberation of insulin from the pancreas is so high that storage in the form of granules does not take place. Degranulation in instances of diabetes should not, therefore, be attributed to hypoinsulinemia but may be taken to indicate an increased rate of release of insulin under the influence of hyperglycemia.[26] Beta cell degranulation in animals made diabetic by partial pancreatectomy or by administration of steroids or growth hormone is, therefore, interpreted as reflecting increased insulin output.

The degranulation of β cells caused by sulfonylurea administration is known to be a direct effect of the drug on the β cells and not a consequence of the depression of blood sugar level.[25,27,36–39] This degranulation is also indicative of increased insulin output.

In other work,[40,41] it has been demonstrated that β cells contain glucose-6-phosphatase and it has been postulated that the rate of insulin production by the β cells depends on the amount of glucose-6-phosphate which is available for β cell metabolism. Since an increased blood sugar level would increase available glucose-6-phosphate this may explain the way in which glucose controls insulin output. Furthermore, it has been shown that there may be some interference with β cell glucose-6-phosphatase activity after sulfonylureas. This may explain the mechanism by which the sulfonylureas increase insulin output by preventing the normal breakdown of glucose-6-phosphate and, therefore, making more available for β cell metabolism despite reduced blood sugar levels.[42]

Additional morphologic changes indicative of increased insulin output from β cells occur in the rabbit but not in the dog. These include, in early stages,

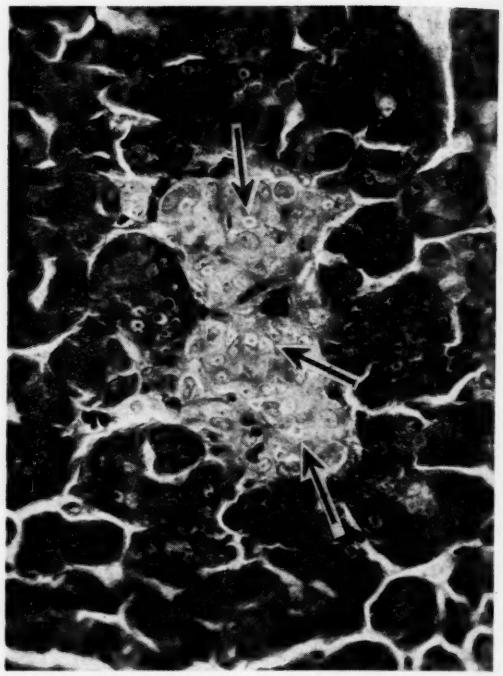


FIG. 8. Pancreas of 50 per cent partially pancreatectomized dog treated with 0.5 gm. of tolbutamide twice daily orally for fifty days. There is β cell degranulation, enlargement of β cell nuclei and Golgi apparatus (arrows), but no other changes. PAS-Trichrome. X 275.

increased mitotic activity of the β cells and later neogenesis of β cells from duct epithelium. These changes seem to account for the temporary nature of the diabetes induced in rabbits by hormonal treatment. The failure of the dog to show adaptation to prolonged hyperglycemia is apparently based on an inability for new β cell production. This dichotomy in response of the pancreas is a species characteristic. Furthermore, it has been demonstrated[43,44] that neither the puppy nor the pregnant bitch developed diabetes after growth hormone, supposedly because the age and physiologic status of the animal are important in determining the ability of the pancreas to respond to a diabetogenic stimulus.

Vacuolization of β cells and duct epithelium as originally described in the diabetic human,[45,47] dog[20] and cat[6,21] was considered to be due to functional exhaustion of the β cells and was thought to be caused by swelling of cells from imbibition of fluid with their eventual disruption. Subsequently, the vacuolization was considered to be due to artifactitious removal of glycogen deposited within duct epithelium and β cells.[48]

More recently, it was shown that in dogs there is another type of β cell vacuolization occurring during hyperglycemia in which the vacuoles contain neither glycogen nor fat and is associated with nuclear pyknosis.[49]

Glycogen vacuolization of duct epithelium and of β cells is observed in all species during prolonged hyperglycemia and does not lead to cell destruction.[15,48,50,63] This type of vacuolization occurs first in duct epithelium and only later in β cells.[14,15] Furthermore, in some species glycogenization of duct epithelium occurs even in the absence of hyperglycemia.[60,62] The conclusion is that this morphologic phenomenon is the result of hyperglycemia per se and is not related to the functional status of the β cells.[53,54] According to this viewpoint pancreatic glycogen deposits in diabetics would be pathogenetically related to those observed in renal tubular epithelium and in heart muscle. The vacuolization of renal tubular epithelium in diabetes was considered a degenerative process until Ehrlich[55] demonstrated that the vacuoles actually contain glycogen. At the present time renal tubular glycogen deposits are considered to be a reversible metabolic phenomenon which is an expression of a high urinary glucose concentration.[56] Furthermore, the increased cardiac glycogen observed in both human and experimental diabetes is accepted as a mere reflection of the elevated glycemic level.[57]

The other type of vacuolization of β cells seen in the canine during growth hormone administration appears to be quite different from that associated with glycogen deposition. This lesion was not found in the rabbits but only in the dogs. It appears to be a stage in the destruction of β cells that occurs during diabetes in the dog. Since destruction of β cells during prolonged diabetes is seen also in the cat but not in other laboratory animals it is assumed that this type of degeneration of β cells is also found in that species.

The hypothesis that vacuolization and destruction of β cells during diabetes in dogs represents functional overstrain of these cells due to increased demand for insulin was originally put forward by Allen[20] and Homans[8,21] and since has been generally accepted. This mechanism is used to explain the pathogenesis of the permanent diabetes produced by various diabetogenic agents in the dog and cat. However, considerable controversy arose as to whether the stimulus leading to functional overstrain was prolonged hypoinsulinism or prolonged hyperglycemia. Lukens and his co-workers[16,18] demonstrated that the regression of hydropic degeneration in the pituitary diabetic cat treated with insulin followed the reduction of the hyperglycemia to normal levels. They showed that undernutrition and phlorizin poisoning also restores normal islet morphology and concluded that the factor common to the development and maintenance of hydropic change is a high blood sugar level. The view that hyperglycemia per se was responsible for the hydropic change which ultimately leads to β cell destruction was further confirmed by the production of this lesion and permanent diabetes in cats by the injection of massive doses of glucose.[1,2,7] From all the evidence, it would seem that the form of vacuolization which we have called "ballooning vacuolization" is a result of hyperglycemia per se. The mechanism whereby hyperglycemia causes the destruction is not known. It seems unlikely that this is due to a functional exhaustion of the β cells. If it were, it would be remarkable, since, as a general rule, excessive stimulation results in cell hypertrophy and hyperplasia rather than destruction. Furthermore, this phenomenon occurs in the dog and not in the rabbit.

Moreover, if functional exhaustion were the mechanism, one would expect tolbutamide, which also stimulates β cells, to reinforce the β cell destructive action of growth-hormone-induced diabetes. Also in no instance was chronic tolbutamide administration associated with vacuolization or destruction of β cells even when administered for eighty-three days, despite morphologic changes including degranulation and enlargement of Golgi apparatus which are those of chronic β cell stimulation.

SUMMARY

Rabbits and dogs have been used in studies of the pathogenesis and functional significance of the various pancreatic structural alterations related to chronic hyperglycemia, induced by various diabetogenic hormones with or without 50 per cent partial pancreatectomy. In addition, the effect of sulfonylurea drugs alone or together with hormonal treatment was studied. It was found that the rabbit shows morphologic lesions indicative of increased insulin output which include degranulation of β cells, enlargement of nuclei and Golgi apparatus, increased mitotic activity of β cells and new formation of β cells from duct epithelium. Also, the rabbit pancreas shows vacuolization of duct epithelium and of β cells due to glycogen infiltration which is associated with hyperglycemia but is not indicative of insulin secretory action. In the dog, chronic hyperglycemia causes β cell degranulation and enlargement of nuclei and Golgi apparatus, and glycogenization. However, no new formation of β cells either by β cell mitoses or by neogenesis from other cell types is

found. Furthermore, in early stages the dog shows a second type of β cell vacuolization containing neither glycogen nor fat but with nuclear pyknosis which is followed by eventual loss of β cells. This latter lesion has been interpreted to be a response to hyperglycemia through some unknown mechanism but not related to the functional status of the β cells. This viewpoint was supported by the fact that chronic stimulation of β cells by sulfa drugs causes degranulation and enlargement of nuclei and Golgi apparatus but no other changes. Finally, pretreatment with sulfonylureas does not measurably alter the pancreatic response of the rabbit or dog to diabetogenic hormones.

SUMMARIO IN INTERLINGUA

Le Effecto de Agentes Insulinogenic Super le Insulas Pancreatic

Conilios e canes esseva usate in studios del pathogenese e del signification functional del varie alterationes in le structura del pancreas le quales es relationate al chronic hyperglycemia inducite per varie hormones diabetogene con o sin pancreatectomia partial de 50 pro cento. In plus, le effecto de sulfonylureas esseva studiate in administration isolate o combinate con tractamento hormonal. Esseva constatate que le conilio exhibi lesiones morphologic de typos indicante un augmento del rendimento de insulina. Iste lesiones include disgranulation de cellulas beta, allargamento del nucleos e del apparato de Golgi, augmento del activitate mitotic del cellulas beta, e neoformation de cellulas beta ab epithelio del ductos. Le pancreas del conilio etiam monstra vacuolisation de epithelio ductal e de cellulas beta como effecto del infiltration de glycogeno que es associate con hyperglycemia sed que non indica un action secretori de insulina. In le can, hyperglycemia chronic causa disgranulation de cellula beta, allargamento de nucleos e apparatos de Golgi, e glycogenisation. Tamen, nulle neoformation de cellulas beta per mitose o per neogenese ab altere typos cellular es incontrate. In plus, in stadios precoce le can monstra un secunde typo de vacuolisation de cellulas beta, non continente glycogeno o grassia sed con pycnose nuclear que es sequite in le curso del tempore per perdita de cellulas beta. On ha interpretate iste ultime lesion como un responsa al hyperglycemia, mediate per un non cognoscite mechanismo que non es relationate al stato functional del cellulas beta. Iste conception trovava supporto in le facto que le stimulation chronic de cellulas beta per drogas sulfa causa disgranulation e allargamento de nucleos e de apparatos de Golgi sed nulle altere modification. Finalmente, pretractamento con sulfonylureas non altera mesurabilemente le responsa pancreatic de conilios e canes al action de hormones diabetogene.

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Human Growth Hormone

A Preliminary Report

Philip H. Henneman, M.D., and Dorothy H. Henneman, M.D., Jersey City

INTRODUCTION

The clinical picture of acromegaly has intrigued physicians since the first description by Vicenzo Brigidi in 1877,[1] and the better known description by Pierre Marie in 1891.[2] This natural experiment in hyperpituitarism demonstrated that the human pituitary produced a growth factor effective in adults as well as in children. Despite the striking clinical alterations in acromegalic patients the only major chemical abnormalities consistently recorded in these patients have been the elevated inorganic serum phosphorus and the impaired glucose tolerance with insulin resistance. A frequent paradoxical observation has been the hypercalciuria of acromegalic patients who would be expected to conserve calcium for their obvious new bone formation. A little-emphasized but intriguing question underlying this bizarre clinical picture concerns the physiological role of growth hormone in normal adults. This then is part of the background to which recent studies with human growth hormone (HGH) pertain.[3-9]

METHODS

The demonstration by Knobil in 1954[10a] and 1957[10b] that beef growth hormone was ineffective in monkeys whereas monkey growth hormone was effective indicated that species specificity was probably responsible for the previous failures to demonstrate activity of beef growth hormone in man. Accordingly, collections were begun of human pituitaries obtained at autopsies and from this material human growth hormone was extracted by Drs. Raben,[11] Li,[12] Wilhelmi,[13] and a group in England.[9] At least four different methods of chemical isolation have been devised. Our studies have utilized an acid-soluble extract prepared by Dr. Raben. This preparation is well-tolerated and appears to be free of significant contamination with other anterior pituitary hormones.

The data which follow concern balance studies and measurements of levels of intermediary carbohydrate and fat metabolites in the blood and urine of fourteen patients during both brief and prolonged administration of HGH. Studies have ranged in duration from six days to eighteen months and have been carried out* upon four children with hypopituitarism, one young adult with congenital hypopituitarism, one elderly individual with idiopathic acquired hypopituitarism, an infant with hyperinsulinism,† a markedly obese middle-aged woman, a cachectic man with malignant melanoma, a young woman with gonadal dysgenesis and short stature, a young woman with Addison's disease, and three adult patients with mild diabetes mellitus.‡ The analytical and balance study technics have been described previously.[14,15]

RESULTS: CLINICAL AND BALANCE DATA

1. *Growth.* Growth hormone, 1-2 mg. daily, has been administered to three children with hypopituitarism for nine to eighteen months each. Growth rates in these three cases increased from 0.20, 0.20, and 0.25 cm. per month to 0.35, 1.0, and 0.4 cm. per month respectively. Treatment has been withdrawn in two children for prolonged periods with return to previous growth rates. Growth appears to have been somewhat more rapid the first six months of treatment than the second six months of treatment in each patient. Growth appears to have been somewhat greater during a treatment schedule consisting of HGH 2 mg. daily for "two weeks on" and no treatment for "two weeks off" repeated in a cyclical fashion than the growth rate observed during treatment with HGH 2 mg. every other day without interruption.

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From the Department of Medicine, Seton Hall College of Medicine, Jersey City Medical Center, Jersey City, New Jersey.

*Studies were carried out on the metabolic ward of the Massachusetts General Hospital.

†Studied in cooperation with Dr. John Crigler at the Childrens Medical Center, Boston.

‡Two of these patients were studied on the metabolic ward of the Royal Victoria Hospital, Montreal, in cooperation with Drs. J. C. Beck and E. McGarry.

2. *Balance Data.* The administration of 0.2-10 mg. human growth hormone daily regularly produced retention of nitrogen, phosphorus, sodium, and magnesium in the same proportion as these substances occur in general protoplasm.[24] Nitrogen retention was absent to slight the first day of treatment, reached a maximum the third to fifth days, continued at levels of 2-6 gm. per day for two to three weeks, and in the fourth and fifth weeks of therapy gradually waned so that in the sixth week of therapy nitrogen retention was barely evident in the balance data. In one patient HGH was omitted from the sixth to eighth weeks and then resumed with prompt return of sustained nitrogen retention. On discontinuing growth hormone there was slight negative nitrogen balance, but losses of nitrogen did not approach the quantities retained during HGH. The changes in nitrogen excretion were matched by a progressive fall in the blood urea nitrogen to approximately 50 per cent of control level. On the other hand, serum inorganic phosphorus gradually rose 1 to 2 mg. per 100 ml. during prolonged treatment.

Although potassium retention in general paralleled nitrogen retention, it is noteworthy that potassium retention greater than that predicted from the nitrogen balance occurred the first day on treatment, and disproportionate loss of potassium occurred the first day off therapy. This peculiar response recalls the disproportionate loss and retention of potassium the first day on and the first day off cortisone respectively.

Calcium metabolism deserves special description. During HGH the urinary calcium gradually rose to levels two to three times control. This unexpected increase in urinary calcium appeared to be accounted for by an almost identical simultaneous decrease in fecal calcium, and calcium balance was little altered. Whereas nitrogen retention waned on prolonged HGH administration, hypercalciuria and increased calcium absorption continued unabated. On discontinuing HGH the urinary calcium promptly fell to nearly zero and fecal calcium remained low for at least eighteen days. Thus, marked calcium retention occurred following HGH in contrast to the minimal retention observed on HGH. It should be noted that this pattern of calcium metabolism was observed not only in the hypopituitary children with open epiphyses, but in the adult hypopituitary patient with closed epiphyses and in the adult obese patient with closed epiphyses.

3. *Theoretical Weight Curves.* In careful balance studies on stable patients there is generally an excellent correlation between changes in the weight of the patient and changes in nitrogen, sodium, and potassium balance. During growth hormone administration the "theoretical weight"[24] predicted from the balance data and from the weight changes of the fore-control consistently diverged from the actual weight of the patient. In each case actual weight failed to increase as much as predicted from the balance data. This discrepancy appeared rapidly during the first week of therapy and changed very slowly thereafter.

INTERPRETATIONS OF BALANCE DATA

The observed increased growth rate and retention of nitrogen and other constituents of protoplasm indicate that human growth hormone is effective in man. Special features are retention of nitrogen at a rate far greater than the rate observed in normally growing children and waning of the nitrogen response on prolonged administration. The pattern of calcium metabolism suggests that growth hormone increases calcium absorption but, at least initially, does not promote calcium deposition in bone. This recalls the assay in rats in which growth hormone stimulates chondrogenesis with widening of the epiphyseal plate but, at least initially, produces little evidence of accelerated osteogenesis. It is noted that only after discontinuing HGH does one observe marked calcium retention consistent with active osteogenesis. The nitrogen and calcium findings suggest that intermittent withdrawal of treatment may be more effective than constant therapy and may even simulate the normal pattern of secretion. Finally, the discrepancy between actual and theoretical weight during growth hormone administration suggests either loss of water or loss of fat. That the weight loss in part represents increased fat mobilization and utilization is further suggested by the loss of appetite observed in most of the patients treated with HGH and the improvement in appetite on discontinuing HGH.

RESULTS: CARBOHYDRATE AND FAT METABOLISM

Beck and associates[3] and Ikkos, Luft, and Gemzell[7] demonstrated that human growth hormone in man produced a decreased tolerance to administered glucose. Luft et al.[7] and Pearson et al.[6] noted accelerated production of ketone bodies in diabetic and hypophysectomized diabetic subjects. The degree of ketosis appeared related in some manner to the degree of hypopituitarism as well as to the degree of diabetes.

A. *Effects of* HGH *in Patients with Hypopituitarism, Hyperinsulinism, and Obesity.* 1. HGH produced changes in blood and urinary levels of carbohydrate and fat metabolites which preceded major changes in nitrogen balance by approximately twenty-four hours. Elevations

in fasting levels of plasma NEFA, as previously reported by Raben,[37] were observed four hours after HGH in hypopituitarism in an obese subject on an 800-calorie diet and in an infant with hyperinsulinism. Fasting levels of NEFA and citrate determined twenty-four hours after HGH also increased as the rate of retention of protoplasmic constituents became maximal (third to fifteenth days of HGH).

In hypopituitarism urinary excretion of ketones and citrate increased within twenty-four hours. Increases in ketones were variable thereafter but generally remained above control during maximal nitrogen retention. Control levels of urinary citrate were below normal during the control period; these progressively increased to normal or above normal levels during marked nitrogen retention.

In the obese patient the reduced caloric intake prior to HGH produced increased levels of plasma NEFA, serum citrate, urinary ketones and urinary citrate. As HGH produced maximal retention of protoplasmic constituents, levels of urinary ketones decreased while those of serum and urinary citrate and plasma NEFA rose.

2. No significant changes were observed in levels of blood and urinary glucose, pyruvate, lactate, or alpha-ketoglutarate in the patients with hypopituitarism, or in the infant with hyperinsulinism during either the initial phase (four hours to three days) or the phase of maximal nitrogen retention (three to fifteen days). Fasting, twenty-four-hour post levels of blood glucose and alpha-ketoglutarate rose while those of pyruvate fell in the obese patient during the first two weeks of therapy.

3. After two weeks of HGH, waning retention of protoplasmic constituents was paralleled by a fall to control levels of plasma NEFA and serum and urinary citrate; urinary ketones fell to control in hypopituitarism and decreased further in the obese subject. Blood glucose decreased to control in the obese subject and remained the same or fell below control in the hypopituitary patients. Tolerance to administered glucose decreased in all three hypopituitary patients and was accompanied by an abnormal postglucose elevation in serum citrate in two patients.

B. *Effects of* HGH *in Mild Diabetes.* In mild diabetes, HGH produced marked alterations in levels of fat and carbohydrate metabolites despite normal or totally absent retention of protoplasmic constituents. The three patients studied had minimal diabetes of late onset and were uncomplicated. The degree of hyperglycemia and glycosuria either prior to or during HGH could not be correlated with the degree of nitrogen retention produced by HGH.

In E.L. (male, age sixty-three, controlled by diet alone, normal weight), HGH produced marked retention of protoplasmic constituents. This was accompanied by elevations in fasting plasma NEFA and fasting serum citrate, a normal *fall* in postprandial levels of ketones and citrate, and a decrease in fasting blood and urinary levels of ketones. A marked and progressive increase in both fasting and postprandial glucose and glucosuria accompanied these changes. When HGH was discontinued, levels of glucose, NEFA, and serum citrate returned to control. However, urinary ketones remained below control and urinary citrate levels remained twice normal. There were no significant alterations in pyruvate, lactate, or alpha-ketoglutarate levels. Pertinent data are shown in figures 1 and 2.

In I.P. (female, age forty-three, history of diabetes

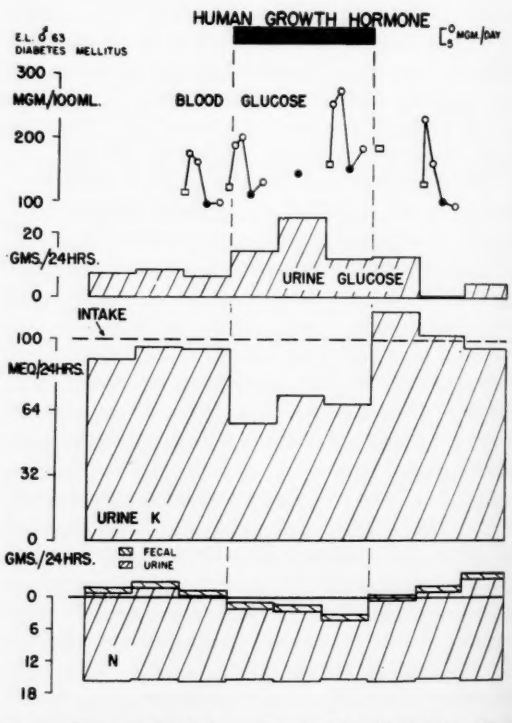

FIG. 1. Effects of HGH in a patient with mild diabetes. Blood samples were taken fasting at 8:00 a.m. (open square), after breakfast at 8:30 and 10:00 a.m. (open circle), at 12 noon (solid circle), and after lunch at 4:00 p.m. (open circle). HGH was injected immediately after the 8:00 a.m. venipuncture. Breakfast consisted of 100 gm. of carbohydrate. The study included three days each of forecontrol, HGH treatment, and postcontrol.

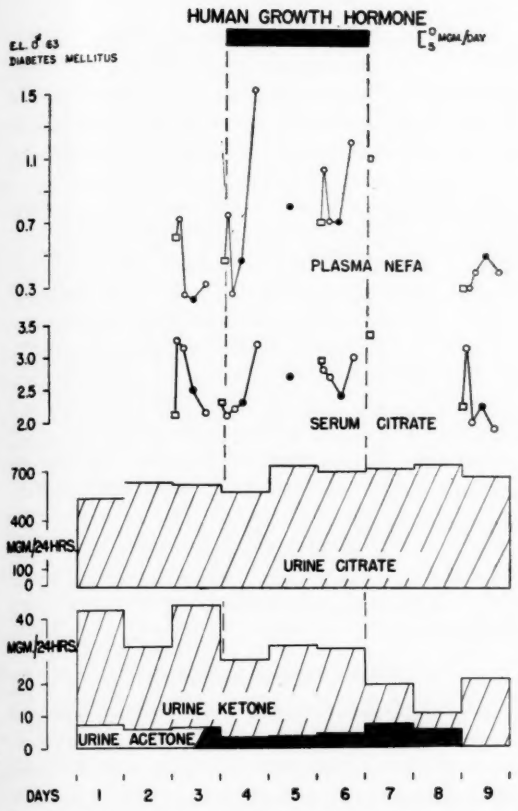

FIG. 2. Effect of HGH in a patient with mild diabetes. Scale for plasma NEFA (nonesterified fatty acids) is milliequivalents per liter and for serum citrate milligrams per 100 ml. Normal values for fasting NEFA are less than 0.7 mEq./L. and for serum citrate less than 2.6 mg. per 100 ml. Normal levels of urinary ketones are less than 20 mg. per twenty-four hours and for urinary citrate less than 480 mg. per twenty-four hours. For details of blood sampling see legend for figure 1.

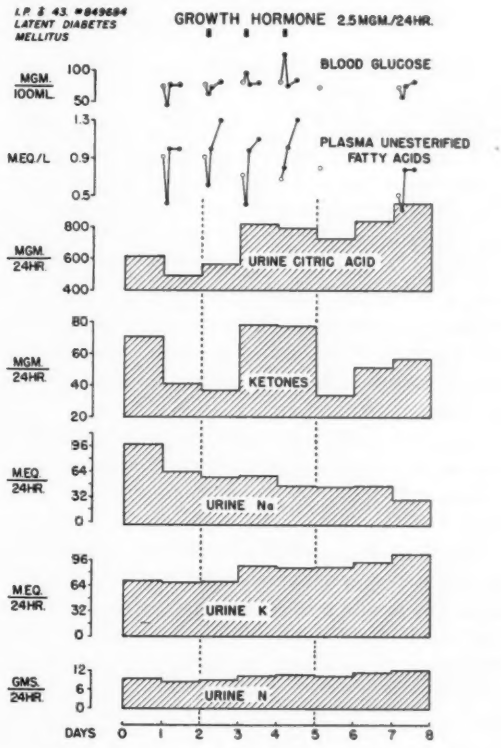

FIG. 3. Effects of HGH in a patient with mild diabetes. Blood values were obtained fasting at 8:00 a.m. (open circles), two and four hours after breakfast and four hours after lunch (solid circles). HGH was injected following the fasting venipuncture. Breakfast was eaten at 8:05 a.m. and lunch at 12:05 p.m.

prior to weight reduction, moderately obese at time of study), HGH produced no retention of protoplasmic constituents. No increase in fasting plasma NEFA or serum citrate was observed, but postprandial plasma NEFA progressively increased as did blood and urinary ketones. Fasting blood glucose showed no change but postprandial concentrations progressively increased. When HGH was discontinued, glucose, NEFA, and ketones returned to or below control values. Urinary citrate, however, continued to increase to twice normal levels during the postcontrol period. Pertinent data are shown in figure 3.

In the third diabetic patient, I.K. (female, age sixty-four, obese, controlled by diet and 8 units of Protamine Zinc Insulin), HGH produced changes which initially were comparable to those observed in E.L., but which thereafter were comparable to those seen in the second patient, I.P. Less than normal retention of nitrogen occurred on the first day accompanied by transient elevations in fasting plasma NEFA and serum citrate and transient decreases in blood and urinary ketones. Thereafter nitrogen retention waned, fasting and postprandial NEFA fell toward control, and urinary ketones gradually increased as did urinary citrate and alpha-ketoglutarate. Postprandial citrate rose in an abnormal fashion. Blood and urinary glucose increased with each dose of HGH and returned to less than control when HGH was discontinued. Urinary ketones, alpha-ketoglutarate, lactate and citrate continued to rise when HGH was discontinued.

INTERPRETATION OF CHANGES IN CARBOHYDRATE AND FAT METABOLITES LEVELS

The prompt and continued increase in plasma NEFA levels produced by HGH in hypopituitarism, hyperinsulinism, and simple obesity suggests that this hormone may accelerate the mobilization of fat. The ketonuria produced in hypopituitarism further suggests an increase in the availability of two-carbon fragments with the rate of production exceeding the rate of disposal of acetyl Co A. Increases in serum and urinary citrate suggest alterations in the tricarboxylic acid cycle though the specific mechanism remains obscure. The direction and sequence of changes in potassium, sodium or calcium balances during HGH do not explain the changes in citrate.[18]

The shift from very marked to slight nitrogen retention in the patients with hypopituitarism or in the patient with obesity does not appear related to insulin lack.

The changes in blood and urinary glucose, citrate, and ketone levels observed in these patients during the phase of waning retention were not comparable to those observed in the diabetic patients in whom minimal or absent nitrogen retention occurred.

Milman et al.[19] and Lukens[20] demonstrated in animals that insulin was essential for the anabolic effect of growth hormone. This appears to be true in man also in view of absent or minimal nitrogen retention in two of three mild diabetics. It is to be especially noted that although the anabolic effect was impaired by minimal insulin lack, a marked effect on blood and urinary levels of glucose, citrate, alpha-ketoglutarate, and ketones was still present.

This may reflect, in part, the increase in glucose production reported by Altszuler et al.[21] when growth hormone is administered to hypophysectomized dogs.

Under certain circumstances growth hormone appears to increase the secretion of endogenous insulin.[19,22] The evidence which suggests that HGH in man may produce a similar increase in insulin secretion is as follows: (1) Ketone levels decreased from above normal to normal in the obese subject on an 800-calorie diet and in the diabetic patient who showed normal nitrogen retention during HGH; (2) blood and urinary glucose fell below control in this same diabetic subject when growth hormone was discontinued; and (3) fasting levels of plasma NEFA, and serum citrate and urinary ketones and citrate fell below control during prolonged administration or following its discontinuation in the patients with hypopituitarism.

SUMMARY

Human growth hormone (HGH as prepared by Raben) is well tolerated and appears free of other hormones. Growth rate is stimulated in hypopituitarism and balance studies reveal retention of nitrogen, phosphorus, sodium, and magnesium. Potassium retention is disproportionately large the first day on HGH and loss is disproportionately large the first day off HGH. HGH produces hypercalciuria equal in degree to the observed increase in calcium absorption. Calcium retention is prominent only after HGH is stopped. Theoretical weight changes are consistent with fat mobilization.

Human growth hormone produced a prompt rise in plasma NEFA and serum and urinary citrate and ketones without significant changes in glucose, pyruvate, or lactate in patients with hypopituitarism. Levels of these constituents returned to control as nitrogen retention waned on prolonged HGH administration. Minimal or absent nitrogen retention in two of three patients with mild diabetes was accompanied by evidence of progressive impairment in glucose utilization and abnormal elevations in levels of citrate and alpha-ketoglutarate.

SUMMARIO IN INTERLINGUA

Hormon de Crescentia Human: Un Reporto Preliminar

Hormon de crescentia human (HGH, preparate secundo Raben) es ben tolerate e pare esser libere de altere hormones. Le crescentia es stimulate in hypopituitarismo, e studios de balancia revela retention de nitrogeno, phosphoro, natrium, e magnesium. Le retention de kalium es disproportionalmente grande le prime die del administration de HGH, e le perdita de kalium es disproportionalmente grande le prime die del re-suspension de HGH. HGH produce hypercalciuria equal in grado al observate augmento del absorption de calcium. Le retention de calcium es prominente solmente post le suspension de HGH. Le alterationes theoric del peso es in congruentia con le mobilisation de grassia.

HGH produceva un prompte augmento del non-esterificate acido grasse del plasma e del citrato e cetones del sero sin significative alterationes de glucosa, pyruvato, o lactato in patientes con hypopituitarismo. Le nivellos de iste constituentes retornava al valores de controlo in tanto que le retention de nitrogeno declinava con un prolongate administration de HGH. Grados minimal de retention de nitrogeno o le complete absentia de illo in duo inter tres patientes con leve diabete esseva accompaniate de evidentia de un progressive vitiation del utilisation de glucosa e de elevationes anormal del nivellos de citrato e de alpha-cetoglutarato.

ACKNOWLEDGMENT

This work was supported by United States Public Health Service Research Grants #A-2970, #A-1661, #A-2971, #A-605, by the Ayerst Laboratories and by a grant from the office of the Surgeon General, Department of the Army (Control DA-49-007-MD-798).

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Glucagon and Metaglucagon Diabetes in Rabbits

J. Logothetopoulos, M.D., B. B. Sharma, M.D., J. M. Salter, Ph.D., and C. H. Best, M.D., Toronto

Early attempts in this and other laboratories to demonstrate a diabetogenic action of glucagon have met with limited success. It was found that in order to produce a temporary diabetes with glucagon it was necessary either to force-feed[1,2] the animals or to administer cortisone simultaneously.[3]

Investigations described here differ from previous ones in that glucagon administration began on the second postnatal day and was continued for many months. Our purpose was to obtain additional information concerning the atrophy of alpha cells which follows prolonged treatment with glucagon.[4] We were, however, surprised to find that the anorexia produced by glucagon in adult rabbits did not occur in immature animals. The treated rabbits ate as much and grew nearly as well as their littermate controls. By the end of ten weeks seven of the nine glucagon-treated rabbits had blood sugar levels of about 300 mg. per 100 ml. throughout the day and were excreting 5 to 15 gm. of glucose daily. Continuation of the treatment caused a slow but progressive aggravation of the diabetic condition in five; in these cases, after the last injection of glucagon, the diabetes persisted for 63, 50, 30, 20 and 18 days, respectively. A preliminary account of these findings has been published elsewhere.[5]

The results of additional studies, which confirm and extend our original observations, are presented here.

METHODS AND PROCEDURES

Litters of albino rabbits of mixed origin were used. The injections of glucagon started on the second postnatal day. A total daily dose of 2 mg. of glucagon per kilogram of body weight was given at seven to ten-hour intervals. The glucagon was suspended in sterile saline containing about 1,000 units of penicillin per milliliter. Saline containing penicillin was injected in the control animals. The young rabbits were weaned between the fortieth and fifty-fifth days and placed in individual metabolism cages. The food consumption and urine volume were measured daily, and every ten to fifteen days blood sugar levels were estimated at intervals throughout the day. The concentrations of glucose in urine and blood were determined by the Benedict[6] and Somogyi[7] technics respectively. The classical staining technics of Gomori[8,9] as well as the Masson-trichrome method in pancreas fixed in Bouin's and Zenker-formol solutions were used for the investigation of the alpha and beta cells of the islets. Sections from pancreas fixed in Gendre's solutions at —10° C. were stained with the Periodic Acid Schiff method.

Sections of unfixed pancreatic tissue cut at 7.5 μ with a cold knife technic were mounted in glycerin and examined by dark field illumination. In a background of slightly refracting nuclei of β cells and acinar cells, the α cells of control rabbits appear very prominent with their cytoplasm filled with white-silvery granules (figure 1).

To secure an estimate of islet tissue concentration a ratio of islet to acinar tissue area was determined planimetrically on random sections of a piece of pancreas taken from the center of the splenic portion.

A histological study was also made of specimens taken from practically all organs, including endocrine glands.

RESULTS

Transient hyperglycemia, of varying degree and duration, followed each injection of glucagon during the initial phase. The blood sugar level always returned to normal between injections. Figure A illustrates the patterns of hyperglycemia and glucosuria observed in individual rabbits when the transition from intermittent to sustained hyperglycemia was occurring. It can be seen that two of the four littermates which had been treated for ten weeks had already developed a sustained hyperglycemia and severe glucosuria, a third was showing intermittent hyperglycemia and moderate glucosuria, and the fourth had become definitely resistant to the injections of glucagon.

Presented at the Symposium on Changing Basic and Clinical Concepts of Diabetes Mellitus sponsored by The Clinical Society of the New York Diabetes Association, Inc., on Oct. 16, 1959.

From the Banting and Best Department of Medical Research, University of Toronto, Toronto, Canada.

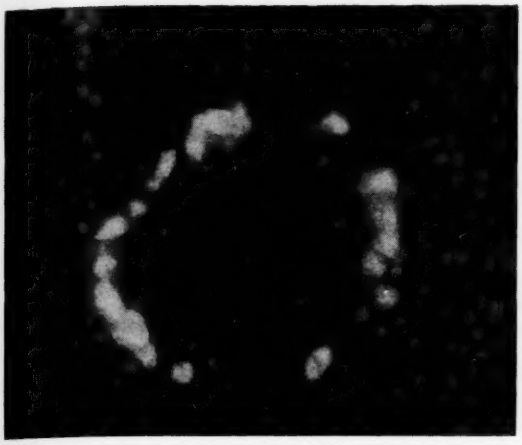

FIG. 1. Pancreatic islet of control rabbit. Islet is delineated by α cells containing highly refractile granules. Fresh-frozen section, mounted in glycerine. Dark-field illumination x 150.

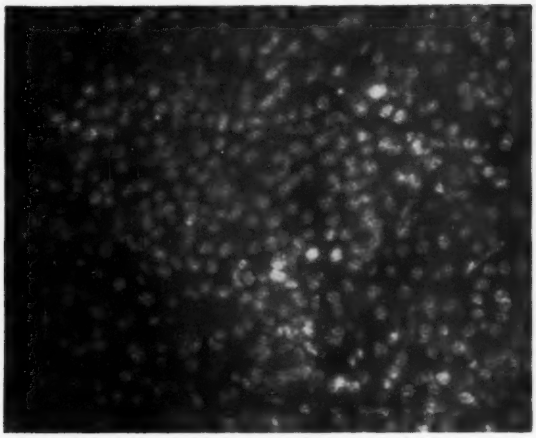

FIG. 2. Pancreatic islet of a rabbit treated with glucagon for ten weeks since third postnatal day. Complete absence of granular α cells. Fresh-frozen section mounted in glycerine. Dark-field illumination x 130.

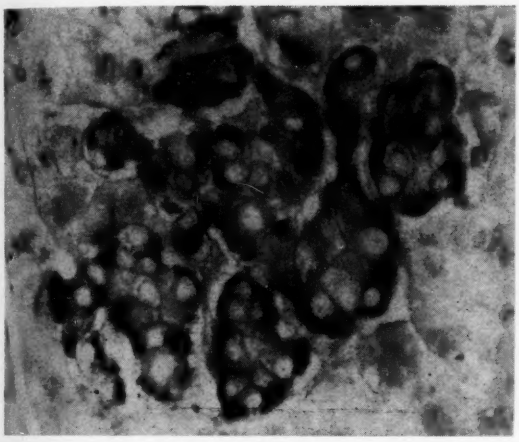

FIG. 3. Pancreatic islet of control rabbit ten weeks old. Normal granulation of β cells. Aldehyde fuchsin-Ponceau 2R x 242.

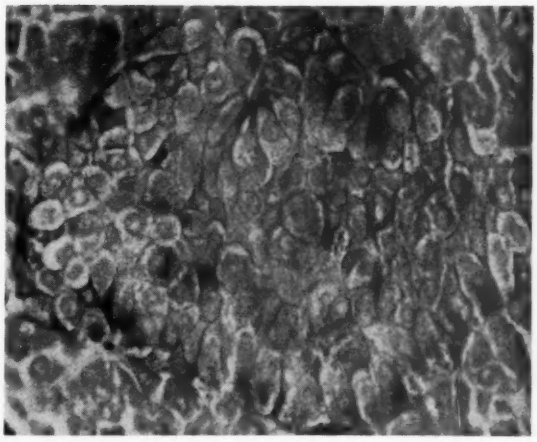

FIG. 4. Pancreatic islet of a rabbit treated with glucagon for ten weeks since second postnatal day. Partial degranulation of closely packed β cells. Aldehyde fuchsin-Ponceau 2R x 193.

The pancreases of the treated rabbits killed during the initial phase (fourth to eighth week) and during the transition period (eighth to twelfth week) showed an increased concentration of islet tissue, partial degranulation and hypertrophy of the β cells and a marked atrophy of the α cells.

The increased concentration of islet tissue was due to an increased number of islets and to an enlargement of individual islets. The rabbits which had become resistant to the hyperglycemic effects of glucagon showed an equally marked hyperplasia of islet tissue. In some cases (figure A) this was more pronounced than in the animals exhibiting severe hyperglycemia.

The degranulation of the β cells was of varying degree and did not appear to be correlated with the severity of the hyperglycemic effect. The extreme atrophy of α cells made it impossible to distinguish them from marginal endothelial or connective tissue cells. Even from the fifth week of treatment many sections from the pancreas had to be searched to find a few α cells retaining specific staining characteristics (figure 6).

Glycogen in the form of fine granules was found in the cells of the islets of rabbits which had just developed a sustained hyperglycemia (figure 8). The density varied

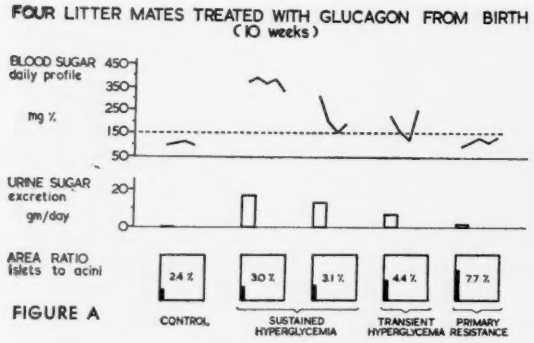

FIGURE A

in individual cells, and one could not be absolutely sure about a definite polarity toward the vascular pole. Glycogen infiltration was also found in cells of the ductules of the pancreas, in a few centro-acinar cells and in the ascending portions of the Henle loops and the distal tubules of the kidney.

Thus far, nine rabbits have been observed for periods of more than three months. Two remained resistant to glucagon treatment. Of the seven which entered the phase of sustained hyperglycemia, two showed a period of decreased food intake, after which they could not be brought back to the previous diabetic level by continued administration of glucagon. In the other five rabbits there was a progressive aggravation of the diabetes. All these rabbits were kept for five to seven months before glucagon treatment was stopped.

The growth rate varied in the glucagon treated rabbits and was on the average slightly less than in the controls. The loss of glucose in the urine was fully compensated by the increased food intake (figure B).

The diabetic animals had severe polyuria and polydipsia. The Rothera test for ketone bodies was occasionally positive during the last weeks of treatment. The progress of metaglucagon diabetes is shown in figure C. Zero day represents the day on which the last injection of glucagon was given. There was no dramatic change in the diabetic state when the glucagon injections were stopped.

The first and second rabbits were sacrificed on the thirtieth and sixty-third day when the diabetic state was subsiding. A biopsy from the pancreas was taken from the third rabbit on the fifty-third day and the animal was then allowed to survive for one month and a half, before it was sacrificed. The fourth and fifth rabbits had short periods of "metaglucagon" diabetes. In both, diabetes was re-established when glucagon treatment was resumed. Ratios of islet to acinar area in the first three rabbits were smaller than in controls.

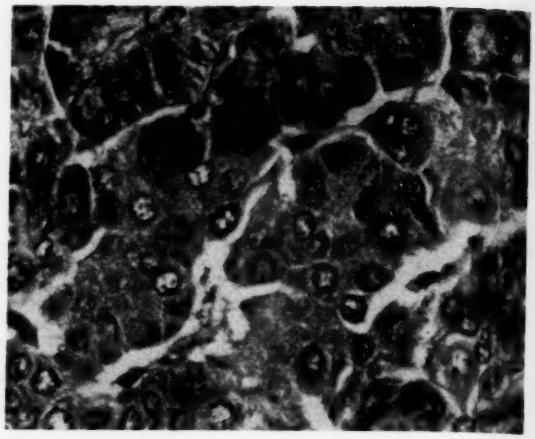

FIG. 5. Pancreatic islet of control rabbit ten weeks old. A row of normal α cells at the periphery. Fixation in Bouin's fluid. Masson's trichrome x 260.

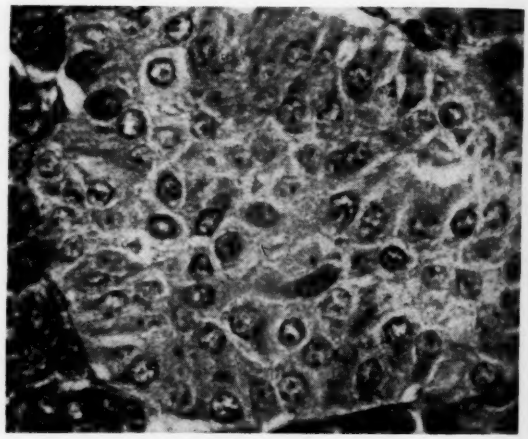

FIG. 6. Pancreatic islet of a rabbit treated with glucagon for ten weeks since second postnatal day. Two atrophic α cells in the upper corner retaining exceptionally good cytoplasmic staining properties. Fixation in Bouin's fluid. Masson's trichrome x 260.

The histological changes in the pancreases removed from the first and second rabbits when sacrificed were the same as those found in the biopsy specimen taken from the third animal on the fifty-third day after the cessation of glucagon injections.

Most islet cells appeared vacuolated and very few had a homogeneous cytoplasm (figures 9, 10). Aldehyde-fuchsin stained granules were occasionally seen in the cytoplasmic rims of the vacuolated cells (figure 10). The islet cells, however, which appeared vacuolated were found to be filled with masses of glycogen when sections from pancreas were fixed in alcoholic picric acid

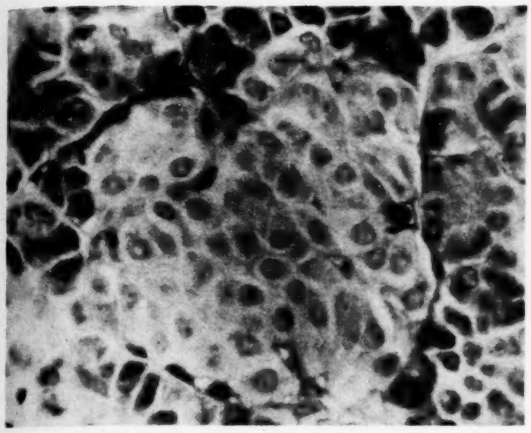

FIG. 7. Pancreatic islet of control rabbit ten weeks old. Periodic Acid Schiff-Haemalum x 265.

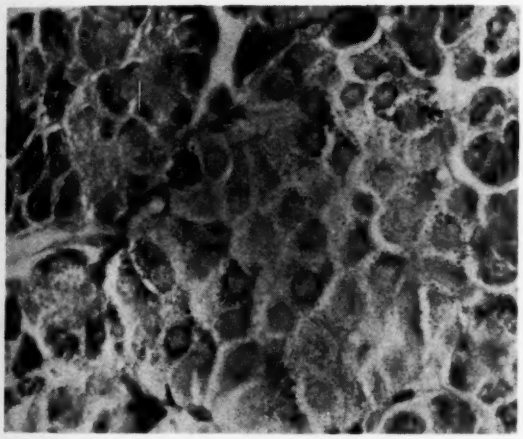

FIG. 8. Pancreatic islet of glucagon treated rabbit with persistent hyperglycemia. Fine granules of glycogen in the cytoplasm of most β cells. Periodic Acid Schiff-Haemalum x 265.

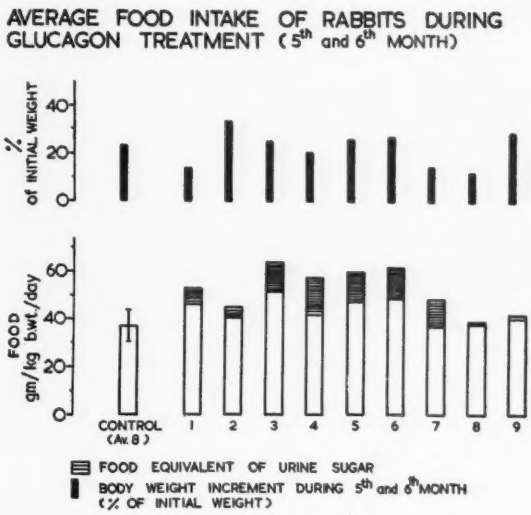
AVERAGE FOOD INTAKE OF RABBITS DURING GLUCAGON TREATMENT (5th and 6th MONTH)
% of INITIAL WEIGHT
FOOD gm/kg b.wt./day
CONTROL (Av. 8)
1 2 3 4 5 6 7 8 9
FOOD EQUIVALENT OF URINE SUGAR
BODY WEIGHT INCREMENT DURING 5th and 6th MONTH (% OF INITIAL WEIGHT)

FIGURE B

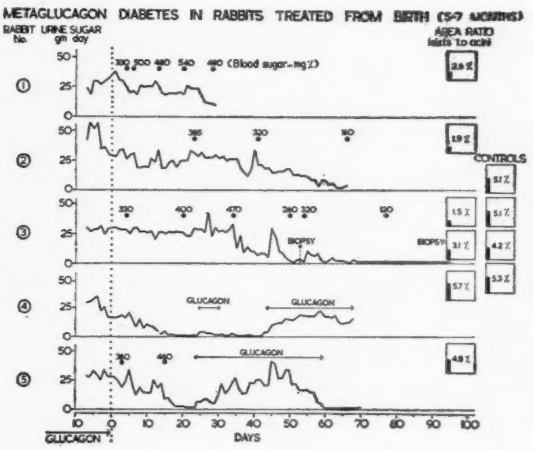

FIGURE C

and stained by the Periodic Acid Schiff technic (figures 11, 12). Digestion with diastase brought back the vacuolated appearance. Glycogen was found also in cells of the ducts and ductules of the pancreas, in centro-acinar cells and in the kidney.

The histological appearance of the islets of two other rabbits sacrificed during glucagon treatment was similar to the one described.

The β cells of Rabbit 3, sacrificed six weeks after the biopsy had been taken, were free of glycogen and fully granulated. Normal β cells free of glycogen and fully granulated were also found in Rabbit 5, killed ten days after glucagon had to be stopped because of a sudden loss of appetite. The food consumption of this animal remained low until it was sacrificed.

Identifiable α cells were not seen for the first three or four weeks, after the cessation of treatment. They started to appear by the fourth and fifth week but never seemed to be as numerous as they were in the controls.

DISCUSSION

The duration and magnitude of the hyperglycemia following each injection of glucagon depends on many factors of which the nutritional state of the animals and compensatory insulin release appear to be very important.

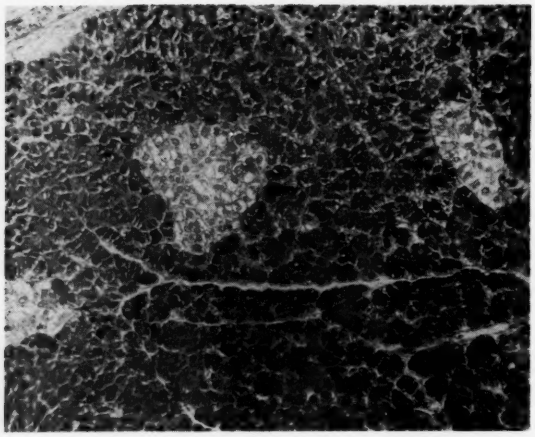

FIG. 9. Metaglucagon diabetes. Thirty days after the cessation of glucagon treatment. Islet cells appear hydropic and vacuolated. Fixation in Bouin's fluid. Aldehyde fuchsin-Ponceau 2R x 60.

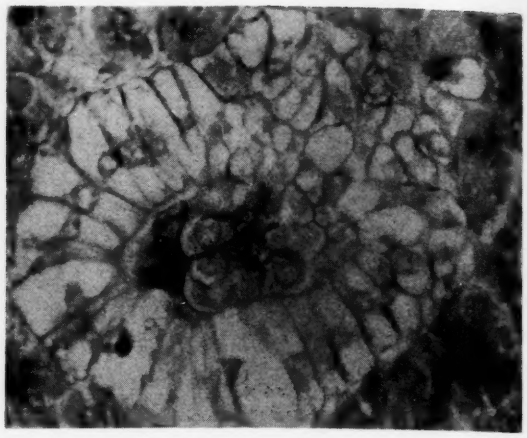

FIG. 10. An islet from the pancreas shown in figure 9. The majority of β cells appear hydropic. Small refractile spherules are seen in some vacuoles. A small group of β cells in the center preserves a normal cytoplasmic appearance and granulation. Fixation in Bouin's fluid. Aldehyde fuchsin-Ponceau 2R x 242.

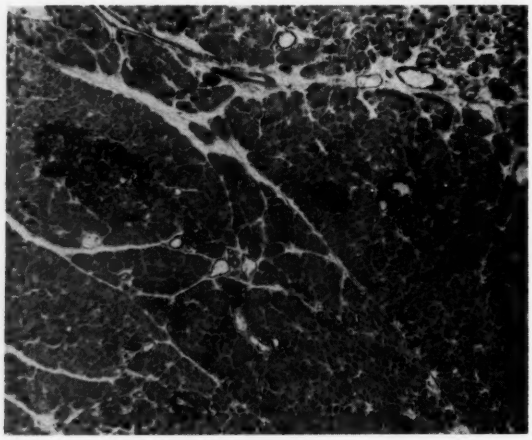

FIG. 11. Islets of the same pancreas shown in figures 9 and 10. Islet cells are filled with glycogen. Fixation in Gendre's fluid at —10° C. Periodic Acid Schiff-Haemalum x 46.

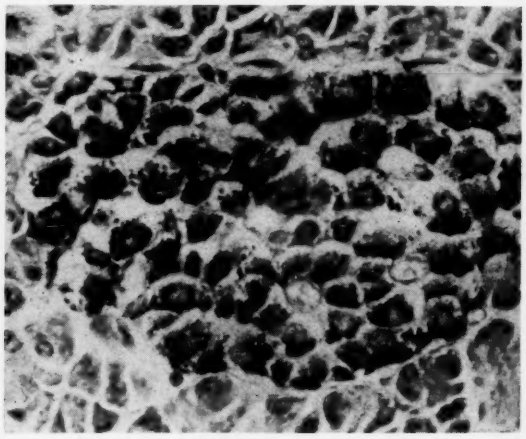

FIG. 12. Pancreatic islet (biopsy) of a rabbit with diabetes persisting fifty-three days after the cessation of glucagon treatment. Glycogen deposition exhibiting "fixation shift." Fixation in Gendre's fluid at —10° C. Periodic Acid Schiff-Haemalum x 236.

The eventual fusion of hyperglycemic waves into a sustained hyperglycemia, observed in some of the rabbits, may be explained by the ultimate failure of the animals to produce adequate amounts of insulin. It would appear that a relative lack of insulin may be established, but, because of the high level of glucose in the extracellular fluids, the over-all glucose utilization seemed to be maintained at a normal level. A similar metabolic state has been reported in guinea pigs treated with cortisone.[10]

Evidence for a peripheral anti-insulin effect of glucagon is controversial,[11] and an inhibitory effect on the activity of the β cells has not been demonstrated. The experiments of Dohan and Lukens,[12] of Brown et al.,[13] leave no doubt that hyperglycemia per se may eventually have a deleterious effect on the islets of Langerhans in many species. A reduced insulin secretion, that would follow as a consequence, would in turn aggravate the hyperglycemia. This cycle may be operating in the "metaglucagon" diabetic rabbits when glucagon injections have been discontinued and endogenous glucagon production has been suppressed. An interesting possi-

bility is that glycogen infiltration of the β cells may be a factor in the vicious cycle. It appeared quite early and paralleled the severity of the diabetes. The glycogen infiltration in the beta cells may not be an innocuous deposition comparable to glycogen deposition in the liver, muscle or in cells of the pancreatic ducts. In the advanced stages of the infiltration with glycogen there may be a mechanical interference with the function of the β cells.

In the "glucagon" and "metaglucagon" diabetic rabbits the β cells were the only endocrine tissue showing this glycogen deposition. The physicochemical factors involved in this phenomenon are poorly understood and merit further investigation.[34,35]

The three rabbits with the prolonged periods of metaglucagon diabetes had a low islet concentration compared with the untreated controls. It is difficult to determine by histological examination whether this was the outcome of a slow progressive loss of β cells or was due to a failure of the formation of new β cells. Whatever the cause the process is extremely slow, compared with the rapid destruction of β cells in dogs treated with growth hormone.[36,37]

The failure of many glucagon-treated animals to develop a sustained hyperglycemia or even to maintain intermittent hyperglycemia may be attributed either to an effective compensatory hyperplasia of islet tissue or to a progressive loss of sensitivity to glucagon. The fact that all resistant rabbits had a high degree of islet hyperplasia favors the first view. Atrophy of α cells occurred during the first weeks of glucagon treatment and persisted throughout the period of treatment. We are unable to state whether they disappear, fail to grow, or become inconspicuous because of shrinking and degranulation. The observation that the α cells were apparently the only ones in the body which atrophied suggests that it was a true compensatory change and supports the view that they manufacture glucagon. Since the argentaffin cells of the gastrointestinal tract were fully granulated and well preserved, it seems unlikely that they possess metabolic characteristics similar to those of the α cells.

The atrophy of the α cells was unrelated to hyperglycemia. It was equally prominent in the resistant and the diabetic rabbits. The reappearance of α cells on cessation of treatment was a very slow process.

As has been previously reported the islets of alloxan diabetic rabbits contain a large number of α cells.[38] This was strikingly demonstrated in fresh-frozen sections examined under dark field illumination. Agglomerates of fully granulated α cells made up most of the islets (figure 15). In contrast the islets of the metaglucagon diabetic rabbits were free from identifiable α cells during the first weeks after cessation of treatment (figure 14), yet the severity of the diabetes in these animals was comparable to that of the alloxan diabetic. The significance of α to β cell ratios in diabetic states becomes very doubtful as a result of these observations.

In conclusion we wish to stress that the experiments described do not provide evidence for or against the role of glucagon in human diabetes. The glucagon-induced diabetes in young rabbits, however, may become a useful experimental tool for the investigation of some basic problems in experimental diabetes. It is quite possible that the prolonged metaglucagon diabetes may be made permanent by intensified treatment of susceptible animals.

SUMMARY

Rabbits were injected subcutaneously at eight-hour intervals with a total daily dose of 2 mg. crystalline glucagon per kilogram body weight. Treatment commenced on the second postnatal day and continued for periods up to seven months. Initially the rabbits had intermittent hyperglycemia and mild glucosuria but after eight to twelve weeks about 40 per cent of the animals developed sustained hyperglycemia and severe glucosuria. The remaining animals became refractory to glucagon treatment. The diabetes grew progressively worse in five of the seven rabbits that were treated continuously for five to seven month periods. When glucagon treatment was stopped the diabetes persisted in these five rabbits for periods varying from eighteen to sixty-three days. The following observations were made:

1. The growth rate of the glucagon-treated rabbits was slightly less than that of the controls. The diabetic rabbits during and after the injections of glucagon compensated for the loss of glucose in the urine by consuming more food.

2. Hyperplasia of the islets, partial degranulation and hypertrophy of the β cells and a marked atrophy of α cells was seen in all treated rabbits, refractory and diabetic alike.

3. Glycogen-deposition in the β cells was first evident at the time the sustained hyperglycemia was developing. In the diabetic rabbits glycogen eventually filled most of the cytoplasm of the β cells. In the two rabbits killed when the severity of the metaglucagon diabetes seemed to subside sixty-three and thirty days after the last injection of glucagon and in the pancreatic biopsy taken from one of the rabbits on the fifty-third day of the metaglucagon period the infiltration of the ma-

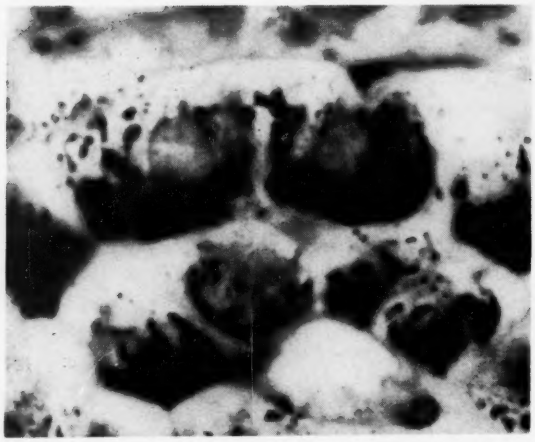

FIG. 13. High power view of upper right portion of islet in figure 12 x 734.

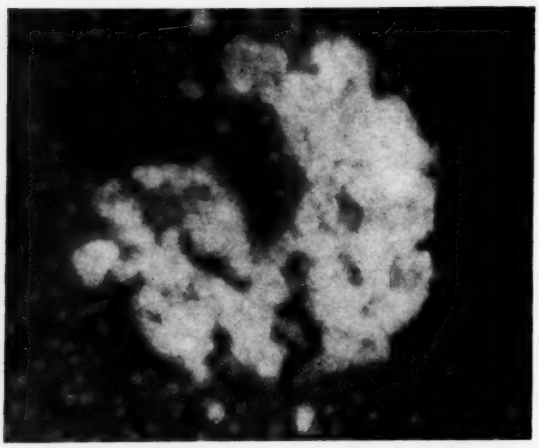

FIG. 15. Pancreatic islet of an alloxan diabetic rabbit. Large agglomeration of fully granulated α cells. Fresh-frozen section. Dark-field illumination x 131.

jority of β cells with glycogen was still extensive. These three rabbits showed also a decreased concentration of islet tissue in comparison with the controls.

4. The α cells remained inconspicuous throughout the period of glucagon treatment and seemed to recover their normal appearance extremely slowly when the injections were stopped.

SUMMARIO IN INTERLINGUA

Diabete per Glucagon e Metaglucagon in Conilios

Conilios esseva tractate a intervallos de octo horas con injectiones subcutanee de glucagon crystallin in un dosage amontante a 2 mg per die per kg de peso corporee. Le tractamento esseva comenciate le secunde

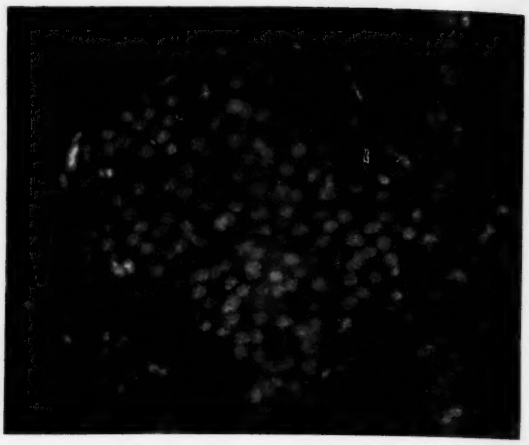

FIG. 14. Pancreatic islet of a metaglucagon diabetic rabbit thirty days after cessation of glucagon treatment. Absence of granular α cells. Fresh-frozen section. Dark-field illumination x 118.

die post nato e continuate durante periodos de usque a septe menses. Initialmente le conilios manifestava hyperglycemia de forma intermittente e glucosuria de leve grados, sed post octo a dece-duo septimanas, 40 pro cento del animales disveloppava hyperglycemia sustenite e glucosuria sever. Le remanente animales deveniva refractori al tractamento con glucagon. Le diabete se faceva progressivemente pejor in cinque del septe conilios tractate continuemente durante periodos de inter cinque e septe menses. Quando le tractamento a glucagon esseva suspendite, le diabete persisteva in iste cinque conilios durante periodos de inter dece-octo e sexanta-tres dies. Le sequente constatationes esseva facite:

1. Le crescentia del glucagonate conilios esseva levemente retardate in comparation con le animales de controlo. Durante e post le injectiones de glucagon, le conilios con diabete compensava le perdita urinari de glucosa per un plus alte consumo de alimentos.

2. Hyperplasia del insulas, disgranulation partial e hypertrophia del cellulas beta, e un marcate atrophia del cellulas alpha esseva trovate in omne le glucagonate conilios, tanto illos que esseva refractori como etiam illos que habeva devenite diabetic.

3. Le deposition de glycogeno in le cellulas beta esseva evidente initialmente al tempore quando le sustenite hyperglycemia esseva in progresso. In le conilios con diabete, glycogeno replenava in le curso del tempore le plus grande parte del cytoplasma del cellulas beta. In duo conilios que esseva sacrificate quando—sexanta-tres e trenta dies, respectivemente, post le ultime injection de glucagon—le severitate del diabete metaglucagonic

pareva subsider e in le specimen bioptic prendite ab un del conilios le cinquanta-tertie die del periodo de metaglucagon, le infiltration del majoritate del cellulas beta con glycogeno esseva ancora extense. Omne iste tres conilios exhibiva etiam un reducite concentration del tissu insular in comparation con le animales de controlo.

4. Le cellulas alpha remaneva inconspicue durante le periodo del tractamento con glucagon e pareva restablir lor apparentia normal lentissimemente quando le injectiones esseva suspendite.

ACKNOWLEDGMENT

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Human Liver Glycogen Levels

The level of liver glycogen, the major carbohydrate store of the body, depends upon the immediate nutritional history of the individual, providing there is no abnormality in the structure of the glycogen or in glycogen production. While numerous values are given in the literature for the glycogen content of the liver of most experimental animals, information on the glycogen content of the livers of normal humans is not nearly as extensive.

Recently, however, J. E. Sokal and K. E. Gerszi (*J. Lab. Clin. Med.* 53:876, 1959) have reported on the glycogen contents of the livers of thirty-seven individuals.

. . . MacIntyre and associates and Young and coworkers obtained liver samples during biopsy while surgical procedures were being carried out on anesthetized patients, while Hildes and associates obtained the specimens by needle biopsy from unanesthetized individuals. It should be noted that all investigators subjected the patients to at least an overnight fast before obtaining the biopsies. From the combined series of data it appears that under these conditions of moderate fasting the upper limit of liver glycogen is about 6 per cent.

Although it has been reported that levels of glycogen in individuals with glycogen storage disease can go as high as 12 to 16 per cent, most of the values are below such a level and there may be considerable overlapping between the glycogen levels in this disease and individuals with normal liver glycogen. Both MacIntyre and associates and Young and co-workers reported that patients who were given large glucose feedings before obtaining the liver biopsy had glycogen levels ranging from 3.2 to 9.7 per cent.

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Regulation of Blood Glucose

Altered Dynamics in Certain Diabetes-like States

Theodore B. Van Itallie, M.D., and Stanley S. Bergen, Jr., M.D., New York City

Homeostasis of blood glucose is essential to life. For this reason, the safeguards built into the mechanism that acts to keep blood glucose above a certain critical level are both numerous and complex. The necessity for maintaining homeostasis of blood glucose rests upon one basic fact: namely, that the brain ordinarily can use only glucose as a source of energy.[1-2] Ability of glucose to penetrate into brain cells depends almost entirely upon maintenance of an adequate glucose gradient across the cell membrane; in contrast to many other cells of the body, permeability of brain cells to glucose does not require the presence of insulin.[3]

Since the liver, the muscle mass, and the adipose tissue are capable of extracting appreciable amounts of glucose from circulating blood, it is clearly important for the body to have mechanisms whereby the flow of glucose to these tissues can be promptly reduced whenever the supply of carbohydrate is threatened. At the same time, the tissues concerned must have an alternate source of fuel that is rapidly available. Viewed in this perspective, it can be seen that the body sometimes must make itself temporarily "diabetic" in order to preserve the integrity of its central nervous system.

When a superfluity of glucose is present in blood two important adjustments normally take place to increase the rate at which the disposal of blood glucose can be accomplished. They are an increased uptake of glucose by the liver[4] and an increased release of insulin by the pancreas.[5] Release of extra insulin permits an increased rate of entry of glucose into both muscle and adipose tissue. Whether liver cells are similarly sensitive to insulin is still a matter of argument.[6-8] However, it seems that the mere raising of the blood sugar promotes glucose uptake by liver, muscle and adipose tissue, presumably by a mass action effect or, as Himsworth[9] has expressed it, because of a greater "head of pressure."[10]

The blood sugar level in subjects in the basal state is determined by the rate at which glucose is released by the liver into the circulation and by the rate of removal of glucose (1) by brain, (2) by liver, (3) by extrahepatic nonencephalic tissues (largely skeletal muscle, heart and adipose tissue), and (4) in hyperglycemic states, by the kidney.

Information is available about these rates. Approximate values and ranges are summarized in table 1. The individual values have been selected from the literature on carbohydrate physiology and are presented as approximations only and not as precise final figures. Nevertheless, it seems possible from these data to arrive at certain useful generalizations about glucose balance.

In figure 1, three glucose curves are shown. The first (A) is a hypothetical curve describing what might happen in a patient with diabetes of moderate severity following discontinuance of treatment with short-acting insulin. In such an individual the fasting blood sugar might be as low as 80 mg. per cent. However, lack of insulin would preclude significant extrahepatic utilization of glucose at this concentration and only the brain would continue to remove glucose at its usual rate of approximately 5 gm. per hour. At the same time, lack of insulin might depress liver glucokinase activity sufficiently to stimulate increased glucose output by the liver to a rate of the order of 15 gm. per hour. During the first hour, 10 gm. of glucose would enter the glucose space (estimated as 17 L. in a 70 kg. male[21]) and increase its glucose concentration by 60 mg. per cent.

TABLE 1

Glucose balance (gm. per hour in a 70 kg. male)

	Output	Uptake
Liver	15 (0-17)[11-14] (28, in diabetic acidosis)[15]	0 (at 150 mg. per cent concentration of blood glucose)[4,10]
Brain		5 (2.6 - 5.1)[16,17]
Extrahepatic area (excluding brain)		<4[18,19] (<4 - 30)[20]

Presented at the Symposium on Changing Basic and Clinical Concepts of Diabetes Mellitus sponsored by The Clinical Society of the New York Diabetes Association, Inc., on Oct. 16, 1959.

From the Department of Medicine, St. Luke's Hospital and the Institute of Nutrition Sciences, Columbia University, New York, New York.

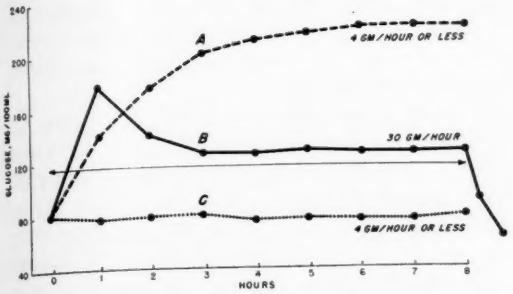

FIG. 1. Relationship of blood glucose concentration and peripheral glucose assimilation rate under varying metabolic circumstances.
A. Glucose curve of hypothetical subject with moderate diabetes following withdrawal of short-acting insulin (basal state).
B. Mean of glucose curves of six normal subjects during eight-hour glucose infusion at 0.5 gm./kg./hr. (Felber, et al.[20]).
C. Glucose curve of a normal subject during prolongation of overnight fast.

There is considerable evidence that, over a wide range of blood glucose levels, glucose uptake is a linear function of the arterial glucose concentration.[22,23] This situation appears to obtain in the diabetic as well as in the normal animal although, in the diabetic subject the "head of pressure" has to be greater than in the normal subject to ensure the same degree of utilization.[9] Within certain limits, the liver uptake or output of glucose also can be considered to be a linear function of the arterial glucose level.[4] Accordingly, as the blood sugar level rises an increased rate of glucose assimilation can be anticipated and the hepatic glucose output would be expected to decrease. Thus, the rising glucose curve would be hyperbolic in shape, flattening out as the utilization rate comes into balance with the decreasing rate of glucose output by the liver. At this new equilibrium, the blood sugar would tend to remain constant. At this level of hyperglycemia, the rate of peripheral glucose assimilation might well be similar to that of a nondiabetic control subject in the basal state.

The second curve (B) is one based on the experiments by Felber et al.[20] involving infusion of glucose into normal subjects for eight hours at a rate of 0.5 gm./kg. body weight per hour. This curve is constructed from the means of data from six experiments and shows that after the third hour of infusion the extrahepatic tissues are able to remove approximately 30 gm. per hour at a blood glucose concentration of less than 130 mg. per cent.

Curve C is the glucose curve obtained in a normal subject whose overnight fast has been extended by eight hours. Evidence from a number of sources indicates that in the basal state only a small fraction of the fuel of the extrahepatic tissues is derived from glucose.[24] Andres and co-workers[19] reported experiments in which the respiratory quotient of forearm muscle in the post-absorptive state was 0.80. They found that even when the forearm was extracting only 0.5 μM. of glucose per 100 mg. tissue per minute, 60 per cent of this amount was accounted for by lactate production from the same site. In these studies, the remaining glucose could account for only 7 per cent of the local oxygen uptake.

Andres and associates[10] calculated that the total glucose uptake for heart and skeletal muscle in subjects in the basal state is approximately 3.6 gm. per hour. Presumably the extrahepatic, extra-encephalic tissues of a normal subject in the basal state do not remove much more than 3 or 4 gm. per hour and may remove less as the period of fasting continues.

From the data in table 1 and figure 1, it can be seen that the capacity of the liver to increase its output of glucose cannot normally exceed the ability of the extrahepatic tissues to remove the released sugar, provided (1) that the normal mechanisms of carbohydrate disposal are intact, and (2) that these mechanisms are given time to adjust to the new glucose load.

On the other hand, when the capacity of the extrahepatic tissues (other than the brain) to accommodate to an increased glucose load is diminished, the liver may fail to decrease its rate of glucose output enough to permit the blood sugar to remain within a normal range. Situations in which a normal fasting blood glucose may be observed in the presence of a grossly impaired disposal mechanism include states of glycogen depletion, such as occur in starvation, and in the glycogen storage diseases.

In diabetes mellitus, hepatic glucokinase activity drops sharply, presumably due to insulin lack, while glucose-6-phosphatase activity increases.[10] Under such circumstances, the liver continues to release glucose even though the concentration of glucose in blood may exceed by far the 150 mg. per cent level that ordinarily is associated with glucose equilibrium across the liver. Apparently the same deficiency of insulin that reduces the ability of the extrahepatic tissues to accommodate to an increased glucose load also may cause the liver to inflict such a load. This makes sense, teleologically, if the propositions are accepted that (1) small quantities of carbohydrate are indispensable to the proper functioning of the Krebs cycle and to maintain muscle gly-

cogen* and (2) that hyperglycemia can partly overcome the block in peripheral glucose assimilation imposed by insulin deficiency.

It is obvious that the hyperglycemia of diabetes mellitus frequently is far from successful as an adaptive measure. When the blood sugar exceeds the renal threshold, glucose is wasted, sometimes in prodigal fashion. Literally, diabetes mellitus as a clinical phenomenon is a "disease of adaptation."

DIABETES-LIKE STATES

The notion of the diabetes-like state has come to be associated with hyperglycemias not primarily due to insulin lack. Such a picture has distinct limitations both etymologically and biochemically. First, the term "diabetes" has nothing directly to do with hyperglycemia or melituria; it refers only to polyuria. Second, modern methods of assay for insulin-like activity so far have failed to document certain clinically clear-cut cases of diabetes mellitus as being due to insulin deficiency.[28] Thus, we are left with the inaccurate term "diabetes-like" and the uncomfortable awareness that the syndrome known as diabetes mellitus is not one but several different diseases.

On the other hand, there are a number of conditions characterized by abnormal hyperglycemia that are clearly not diabetes mellitus. Four of these have been selected for brief discussion and for comparison with what might be called diabetes mellitus proper. These are (1) "hunger diabetes," (2) "glucagon-diabetes," (3) "epinephrine diabetes," and (4) "steroid diabetes."

"Hunger diabetes" is a term that refers to the transient, impaired glucose tolerance and glucosuria that occur after a period of fasting or of carbohydrate privation. It is characterized by a normal or lower than normal fasting blood sugar, an excessive rise in blood glucose after a glucose load, with a subsequent return to control glucose values that is somewhat delayed.[27] Liver glycogen stores are depleted and there is unconfirmed evidence that much of the first supply of glucose ingested after a prolonged fast is stored in the liver rather than being oxidized.[28]

Several theories have been proposed to explain hunger diabetes; it has been variously attributed to temporary failure of insulin secretion in response to hyperglycemia,[29] decreased liver glucokinase activity,[30] and increased secretion of substances rendering peripheral cells relatively impermeable to glucose.[31]

Although the mechanism of hunger diabetes has not been defined, a recent study has shown that the relative assimilation index (an index of peripheral glucose uptake) decreases by approximately 60 per cent in normal subjects given carbohydrate-poor diets for a three-day period.[32] The same study confirmed the observation[33] that net glucose tolerance does not diminish in subjects maintained for several days on diets of which the sole carbohydrate is fructose.

Thus, it would seem that hunger diabetes principally involves impairment of peripheral glucose utilization. Such impairment seems adequately to explain the abnormal glucose tolerance that occurs in this disorder although some diminution of the ability of the liver to remove glucose cannot be ruled out.

The diabetes-like state induced in man when repeated injections of glucagon are given is very similar to hunger diabetes. Again, the fasting blood sugar is normal or low; and again, impairment of peripheral glucose assimilation is noted.

In figure 2A, the response of the blood glucose of a normal subject given a standard breakfast containing 63 gm. of carbohydrate is shown. There is virtually no rise in venous glucose and no glucosuria; in B of the same figure, the response of the same subject to an identical breakfast is shown after three days of repeated glucagon injections (4 mg. intramuscularly every six hours). This time markedly impaired tolerance and glu-

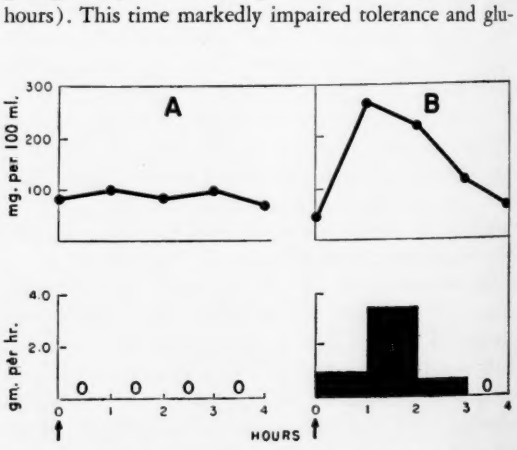

FIG. 2. Changes in blood glucose (mg./100 ml.) and glucosuria (gm./hr.) in a normal subject after breakfast.[34]
A. Before glucagon treatment.
B. During glucagon treatment. Arrows represent ingestion of an identical breakfast containing 63.0 gm. of carbohydrate.

*There is evidence that a certain amount of carbohydrate is required by the tricarboxylic acid cycle for what Peters has called "operative purposes."[24] This seems to be the amount needed to replace the dicarboxylic acids that are lost from the cycle by attrition. Moreover, the muscles require a store of glycogen so that they can continue to function temporarily in situations where energy must be expended under conditions of oxygen deficit.

cosuria occur, even though the fasting blood sugar is slightly lower than in the control test.[34]

It has been proposed that in its early stages at least, the diabetes-like state induced by glucagon is akin to hunger diabetes since fasting blood sugar remains normal or low, liver glycogen is depleted, and the condition can be ameliorated by carbohydrate feeding. Recently it has been reported that a single injection of glucagon administered during the night will be followed by some degree of impairment of the basal glucose tolerance the following morning.[35]

When a diabetes-like state occurs because of a pheochromocytoma it can have all of the characteristics of garden variety diabetes mellitus: fasting hyperglycemia, ketosis and impaired glucose tolerance.[36] This entire picture has been known to disappear following removal of the offending tumor.[37]

Epinephrine is commonly thought of in terms of its hyperglycemic-glycogenolytic effect and it is not widely recognized that epinephrine is a powerful inhibitor of glucose assimilation in the periphery.[38–40]

It has been pointed out that when epinephrine induces glycogenolysis in muscle an increased intracellular concentration of glucose-6-phosphate occurs, which in turn inhibits phosphorylation of glucose.[41,42] In any event, epinephrine "diabetes" also involves a considerable degree of impairment of peripheral glucose assimilation, thereby permitting the hyperglycemia initiated by epinephrine-induced hepatic glycogenolysis to persist.

Last, there is evidence that the glucocorticoids inhibit peripheral glucose assimilation in addition to their other effects.[43–47] Ingle[48] has clearly shown that the intravenous administration of adrenal cortical extract to the eviscerated rat over a period of twenty-four hours decreases its tolerance for glucose given by constant infusion. This effect was observed only in animals treated with insulin.

Although a number of experiments in man and laboratory animals have indicated that the glucocorticoids may interfere with peripheral glucose assimilation, it should be mentioned that DeBodo and Altszuler,[49] in a review published in 1958, concluded that there is no clear-cut evidence of an inhibition of glucose utilization by the adrenocortical steroids.

Certainly, discussion of a possible inhibitory effect on glucose uptake by glucocorticoids should not distract attention from the firmly established action of these hormones upon hepatic glucose production from three-carbon precursors.[50] However, it seems unlikely from what is known about the dynamics of blood glucose regulation that increased hepatic glucose output alone can account for the elevated fasting glucose levels observed so frequently during cortisone treatment and in Cushing's syndrome.

In table 2 an attempt has been made to summarize a few of the gross metabolic characteristics of certain diabetes-like states as they compare with those of classical diabetes mellitus. From this table it can be seen that there are major differences in such parameters as level of fasting blood sugar and liver glycogen content that may help distinguish the disorders from one another; the trait they all share (with the possible exception of steroid diabetes) is impairment of peripheral glucose assimilation.

TABLE 2

Tentative classification of certain diabetes-like states

	Fasting Blood Glucose	Hepatic Glucose Output	Peripheral Glucose Assimilation	Liver Glycogen Content	Ketonemia
DIABETES MELLITUS	↑	↑	↓	↓	↑
"GLUCAGON DIABETES"	N or ↓	↓	↓	↓	↑
"EPINEPHRINE DIABETES"	↑ or N	↑	↓	↓	↑
"HUNGER DIABETES"	↓ or N	↓	↓	↓	↑
"STEROID DIABETES"	N or ↑	↑	N or ↓	↑	N or ↑

It is of course dangerous to generalize about peripheral assimilation of glucose. For example, there is some evidence that epinephrine promotes uptake of glucose by adipose tissue, while it inhibits entry of glucose into muscle.[51,52] The effect of glucocorticoids on peripheral glucose uptake seems quite variable. For the purpose of this discussion, rate of peripheral glucose utilization must be thought of as a "net" value, analogous to the concept of "total peripheral resistance."

It seems helpful to consider the metabolic events that occur in the diabetes-like conditions induced by starvation, glucagon and epinephrine as disorders of glucose conservation. An attempt has been made to show that increased hepatic output of glucose alone is neither a practical nor a feasible way to maintain integrity of the blood glucose level. Even if the liver were able to pour out enough glucose to mimic the effects of a prolonged glucose infusion it would be doing so at enormous cost to the body's stores of protein. In the case of the glucocorticoids, they too seem to be concerned with the need for maintaining glucose homeostasis except that here the emphasis seems to be on increased gluconeogenesis.

SUMMARY

In summary, the relative effectiveness of the liver and the extrahepatic tissues in maintaining glucose homeostasis has been assessed. Analysis of available data leads to the conclusion that peripheral disposal mechanisms normally can accommodate to large glucose loads; hence, it appears that some impairment of peripheral glucose assimilation must be present before a diabetes-like state can occur. This proposition also is consistent with the teleological concept that conservation of glucose, and hence of protein precursors of glucose, is an appropriate metabolic response for the body to make to carbohydrate privation.

SUMMARIO IN INTERLINGUA

Regulation del Glucosa del Sanguine: Alterate Dynamica in Certe Statos Diabetoide

Es evalutate le efficacia relative del hepate e del tissus extrahepatic con respecto al mantenentia del homeostase de glucosa. Le analyse del currentemente disponibile datos resulta in le conclusion que mechanismos peripheric es normalmente capace a adjustar se al demandas de grande cargas de glucosa. Isto significa que le un o le altere vitiation del processo de assimilation peripheric de glucosa debe esser presente ante que un stato diabetoide pote occurrer. Iste these es etiam in congruentia con le concepto teleologic que le conservation de glucosa, e ergo del precursores proteinic de glucosa, es un responsa appropriate del parte del corpore quando illo es private de hydrato de carbon.

ACKNOWLEDGMENT

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Sulfated Polysaccharides and Atherosclerosis

Many lines of investigation are being pursued in man and experimental animals to find a way of preventing, or controlling, the arterial damaging process of atherosclerosis . . . Heparin seems to be effective because it activates a "lipemia-clearing factor" and thus promotes enzymatic lipolysis. A synthetic heparinoid, sulfated alginic acid (SAA), has also been shown to have antilipemic and atherosclerosis-modifying properties in the rabbit. Additionally, it promotes marked lipid phagocytosis in the spleen (N. Gutmann and P. Constantinides, *A.M.A. Arch. Path.* 59:717, 1955). In the present study this synthetic sulfated polysaccharide (SAA) was compared to heparin in respect to its effects on pre-established atherosclerosis in the rabbit (P. Constantinides, P. Saunders, and A. Wood, *A.M.A. Arch. Path.* 62:369, 1956). . . .

SAA also appeared to have a significant effect in retarding the process of coronary atherosclerosis and in reducing the incidence of myocardial necrosis. The effect of SAA differed particularly from that of heparin in that it produced large lipid-laden spleens, while heparin decreased the xanthomatosis of this organ as compared to the control animals. More renal glomerular lipid was also observed in the SAA treated groups, but this appeared to be associated with protection of these animals from renal scarring. Two animals died from massive hemorrhage early in the therapeutic period in each of the treated groups, but reduction in the quantities of administered heparin and SAA eliminated this complication.

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Some Principles of Diabetes Care

T. S. Danowski, M.D., Pittsburgh

I. "MARGINS OF SAFETY" IN REGULATION OF DIABETES

There is a regrettable tendency on the part of young house officers and unindoctrinated physicians to seek refuge in simple "rules of thumb" in treating diabetes without attempting to understand the basic biochemical disorder. This usually takes the form of relating the degree of glucosuria to insulin dosage, i.e., a 4+ is an indication for 20 units of insulin, 3+ for 15, 2+ for 10 units, etc. These simple formulae are filed away in a small black book with other basic information such as the amount of digitalis needed for digitalization, the dosage of belladonna alkaloids necessary for a parasympathomimetic effect, etc. There are several limitations in this approach to treating diabetes: It blocks thought, leaves its custodian leaning on a weak reed, and, most important of all, works just often enough to make its user feel that with a little modification it will provide an easy system of diabetes care. The last of these has, of course, as much substance in fact as the notion that picking the winners on a horse race can be made an exact science. In both instances the basic premise that some relationship between past or current performance and future behavior exists, is sound. It neglects, however, two cogent facts, i.e., that predictions, to be accurate, should take into account all of the variables operative at a particular point in time and that a good correlation on a segment of a curve does not establish that $y = fx$ applies at all other points. A thoughtful physician will not restrict himself by relying upon a narrow mathematical formulation when dealing with a biologic problem such as diabetes. It is far more rational to insist that enough digitalis must be given in a patient with congestive heart failure to obtain a desired effect and that the same principle applies in using atropine or insulin. In our clinic we try to convey this fact by speaking of "margins of safety" in guiding diabetes therapy rather than "rules of thumb." This can perhaps be illustrated most effectively by placing a particular diabetic at a particular time at a definite point on the biologic spectrum of diabetes mellitus (table 1).

The classification of a new diabetic in accord with the scheme shown in table 1 immediately apprises the physician of the existent margins of safety. Thus insulin is not a necessity in the mildest form of diabetes detectable only by strenuous glucose loading, and it is a sine qua non in the last three categories listed. Oral hypoglycemic agents may be tried in the four categories making up the early part of the spectrum but the appearance of ketonuria and ketonemia during such trials indicates that margins of safety are disappearing, that the patient is moving along the spectrum toward ketosis and acidosis and that oral hypoglycemic agents cannot be used in such patients.

How does one select the insulin dosage for patients in the last three categories? By giving an amount which may conceivably produce the desired effect and yet avoid severe hypoglycemia. This is accomplished by the scheme of successive approximations, i.e., 10 units before breakfast and testing the urine for glucose and acetone, 10 units more before lunch if the tests indicate that there has been no therapeutic response, the same before supper, etc. The next day start with 30 units of intermediate or long-acting insulin and continue with successive increments of short-acting insulin before meals as indicated by the results of urine tests.

II. THE DAY-TO-DAY CONTROL OF DIABETES

This is obviously not the task of the physician but of the patient and the family. There are an estimated 3.5 million diabetics in the United States and only 200,000 physicians. If all of them were versed in the management of this disorder, they would each have to assume the responsibility of looking after eighteen patients each day of the year. Actually, since diabetes is a way of life, proper instruction of the patient is the only realistic solution. The emphasis is then shifted from the monthly or less frequent visit to the doctor to the day-to-day decisions made by the patient. The physician steps back and in his traditional and, hopefully, impartial role provides guidance and plans for the

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From the Section of Endocrinology and Metabolism, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, aided by grants from the John A. Hartford Foundation and the United States Public Health Service.

TABLE 1

The diabetes spectrum

Prediabetes →	Latent diabetes →	Simplest form of manifest diabetes →	Obvious diabetes →	Mildly ketotic diabetes →	Acidosis coma (mild) →	Acidosis coma (severe)
(An educated guess based on heredity, obesity, birth weight of offspring.) Rx—Weight reduction by caloric restriction, (?) tolbutamide or biguanide.	(Normal fasting blood sugars and adequate responses to standard G.T.T. but undue hyperglycemia with massive glucose loads, with infections, surgery, etc., and undue hypoglycemia during third, fourth, and fifth hours of a G.T.T.; no ketosis.) Rx—Weight reduction by diabetic diet; tolbutamide or biguanide; if no response: insulin.	(Abnormal standard G.T.T. or undue p.c. hyperglycemia with or without elevated fasting blood sugar levels; no ketosis.) Rx—Diabetic diet; tolbutamide or biguanide; if no response: insulin.	(Fasting hyperglycemia and persistent undue hyperglycemia and usually glycosuria p.c.; no ketosis.) Rx—Diabetic diet; tolbutamide or biguanide; if no response: insulin.	(As in obvious diabetes + ketosis; serum ketone bodies in trace amounts; serum CO_2 content and *p*H within normal range.) Rx—Diet and insulin; *RELIANCE ON ORAL HYPOGLYCEMIC AGENTS UNWARRANTED AND DANGEROUS.*	(Persistent hyperglycemia, glycosuria, marked ketonemia and ketonuria, with lowering of serum CO_2 content to 15 or 10 mEq./L. but with normal *p*H; respirations increased in rate but no Kussmaul breathing; minimal or mild anorexia, nausea, and vomiting.) Rx—Fluids as needed; diet and insulin; *ORAL HYPOGLYCEMIC AGENTS USELESS AT THIS POINT.*	(As in mild acidosis coma but CO_2 content usually less than 10 mEq./L., frequent anorexia, nausea, vomiting and stupor, and *p*H decreased.) Rx—Insulin q.s.ad.; replacement fluids, colloids, potassium and (?) phosphate salts, alkali when *p*H is severely reduced; *ORAL HYPOGLYCEMIC AGENTS USELESS AT THIS POINT.*

prevention and resolution of the complications and concomitants of this disorder. In this post he must function as a pedagogue, a philosopher, and unfortunately, since it must be admitted that in some situations he is quite powerless, as a spiritual adviser. By what principles can he abide in acting out this triumvirate?

III. THE PERIODIC VISIT TO THE PHYSICIAN

The doctor must always try to de-emphasize the importance of the office visit. Every attempt must be made to be sure that the findings at that time are representative of those which prevail when the patient or family is making the decisions. It should be pointed out to the patient that it is deceitful to go into training for the check-up. Several days of more vigorous dieting and of more assiduous attention to urine testing and adjustment of insulin dosages will of course lower the fasting or the postprandial blood sugar and leave the patient and the physician with feelings of mutual approbation. Unfortunately, too often this is followed by relaxation and self-indulgence which then prevail until the time of the next appointment. Also, how much worse is the feeling of frustration if the patient has really tried and at this visit the blood sugar is higher than at the last?

Let us face the issue squarely: Reliance on routine blood sugar as an index of adequacy of regulation is self-deception. If you don't like the sugar level obtained at 8:30 a.m., wait a half hour and perhaps it will be more to your liking. It is well established that significant fluctuations occur with the passage of time. To convince yourself of this, look at the serial readings obtained during control studies run prior to testing the effect of a new oral insulin substitute. Reliance on blood sugar measurements should be reserved for the diagnosis of diabetes, for glucose tolerance tests, for the identification of hyperglycemia, for the demonstration of reduced renal clearances, for proper pre- and postoperative therapy, and for the treatment of diabetic coma. It is not a useful tool, however, in the day-to-day regulation of diabetes.

What of the urine testing record? Here too realism must prevail. Certainly it would be reassuring to know the hour-to-hour ranges of hyperglycemia and glycosuria which prevail, but how many patients can live for their diabetes alone? Since we can't always have five specimens tested a day, let's select those which are most meaningful. In our clinic where we like the simple rationale of combined NPH and short-acting insulin (though not to the exclusion of other insulins or insulin substitutes) the emphasis is placed on the before breakfast (*not the overnight*) and the before supper specimens. The first reflects the adequacy of the dosage of long-acting insulin and the second indicates whether enough, too much, or too little short-acting insulin has been given. However, realism dictates that in some even these two tests a day are an unattainable goal. In these one may have to settle for a set of tests on a working day and another over the week end.

If the measurement of the blood sugar is not the primary goal of the periodic office visit, what is its purpose? First and most important of all it is a time to become acquainted with the patient, with the forces that are making demands upon him, with the defenses

he erects, and with the price he exacts or pays for his adjustments. Second, it is the time to function as a pedagogue and review with the patient again and again the few simple principles which are the basis of diabetes control. It must always be remembered that, try as we will to avoid it, the visit to the doctor is charged with emotion and that communication is always handicapped in an atmosphere of stress. Third, the physician must function as collector of pertinent objective data on the patient. For this the office organization should be geared to periodic evaluation of the patient's status. I believe all of us would agree that knowledge of the patient's lipid levels, electrocardiogram, chest film, the threshold for quantitative vibratory sense perception, the oscillometric index, renal function as reflected in degree of proteinuria, the excretion of dye, the clearance of creatinine should be available at yearly intervals. This can be readily achieved by systematization. In our office, nurses are trained to conduct all of these on a calendar schedule. The cry of "high costs" should be no deterrent. Many patients spend far more in winterizing their car.

Fourth, the doctor must still, unfortunately, function as a philosopher in accepting the end products of a diabetic's lifetime, taking but little credit for the favorable outcome, and forbear punitive attitudes toward the patient or self-accusation when complications or concomitants develop. Finally, when major battles have apparently been lost, the sad role of the consoler must be assumed.

IV. HOW CAN THE PHYSICIAN PREPARE HIMSELF FOR THESE MULTIPLE ROLES?

The emotional impacts and aspects of diabetes upon the patient are of course well recognized. Far less thought, and indeed, credence are given to the emotional involvement of physicians in the problems of diabetes. All of us recognize that certain subjects cannot be discussed without bias. Thus, one can hardly expect to obtain neutral views from a husband about his wife, or from a rabbi, minister or priest about the individual system of religion each represents. In these relationships the emotional involvement is acknowledged to be of such magnitude, and understandably so, that bias weighs heavily in any presentation of views.

And so it is too, but unfortunately with far less justification, when the subject of diabetes is discussed by some physicians.

The emotional involvement of such a doctor in the subjects of diabetes stems in part from a feeling of insecurity in dealing with the many fundamental and practical aspects of diabetes and in part from the need to create in his own mind and in the mind of his patients a system of references which will reassure him and the patient. These may ultimately include such items as faithful reliance on the fasting levels of blood sugar, an unshakable conviction that the degree of diabetes control and the development of vascular disease are inversely related, and premature formulation of opinions on the insulin or chemotherapy of this disorder.

The mechanical aspects of selecting a particular oral insulin substitute, its dosage, the probabilities of response, untoward effects, etc., have been reviewed at intervals and need not concern us here. However, before undertaking the treatment of any diabetic, both the patient and the physician should have a clear orientation of the fundamental biochemical changes in diabetes and their manifestations. In the case of the patient this is necessary because, as has been pointed out, 99 per cent of the time the patient or the family and not the physician is actually treating the diabetes. In the case of the physician, only such knowledge will indicate what is known and what is not known about diabetes, forestall the development of dogma, and make for a serene attitude. In other words it is essential that the doctor be emotionally prepared to acknowledge to himself his woeful lack of sound facts about the unpredictably high incidence of concomitants, or if you will, complications of long-term diabetes. For example, a properly indoctrinated physician who has explored the possibility that a so-called brittle diabetic is not pursuing a reasonable program of life and of diabetes therapy and that factors such as tension, infection, unrecognized shocking are not present, can feel secure that everything possible is being done for the patient and will not turn to or permit frantic behavior. Such an attitude, however, is justifiable only when the doctor knows that he really knows. Similarly, an emotionally uninvolved physician will say that every diabetic patient should be regulated as precisely as possible without exacting an unreasonable price in the way of restrictions upon living, and certainly without shocking the patient. It also follows that in our present state of ignorance no promise can be made that a virtuous life in terms of adherence to an ideal regimen will carry the reward of freedom from vascular disease!

Diabetes presents a limitless field for the exploration of vascular and other diseases and their causes. It is the experiment in nature which can shed light on this aspect of aging in diabetics and nondiabetics and it is here that we who work on the problems of diabetes can make our greatest contribution.

SUMMARIO IN INTERLINGUA

Certe Principios pro le Tractamento del Patiente Diabetic

In su contribution al Symposium Super le Alterationes Progressive del Conceptos Fundamental e Clinic de Diabete Mellite, tenite a New York in octobre 1959 sub le egide del Societate Clinic del Association New Yorkese Contra le Diabete, le ha sublineate le periculo de un mechanisation o schematisation del tractamento de diabete. Ille deplora le frequente methodo de dosar insulina (o su reimplaciamento) exclusivemente super le base del grado de glucosuria presente e insiste super le necessitate de individualisar le tractamento de omne caso particular—un demanda que es rendite minus ardue per le proponimento del autor de rangiar omne caso de diabete in un "spectro" del morbo que ille ha elaborate in le forma de un schema de septe phases ab prediabete usque a sever como acidotic. Le stabilisation continue del diabete in le caso individual non es le responsabilitate del medico sed del patiente e su familia. Un effecto de iste facto es que le medico, quando ille vide le patiente a intervallos periodic, funge non simplemente como un administrator de drogas e tests sed etiam (e super toto) como pedagogo o mesmo como philosopho e curator de anima. Es addite recommendationes specific super le base del experientias del autor in su practica personal.

Vitamin A Tolerance Test and Disorders of Fat Metabolism

Since coronary atherosclerosis accounts for so many deaths in our society, it is fitting that investigators are seeking avidly for a means of detecting some metabolic defect which, in susceptible persons, may provide means for prophylactic management. Some measure of success has been achieved in the statistical relationships between coronary disease and increased blood levels of cholesterol and beta lipoprotein. Further information has come from the use of test meals to measure absorption and utilization of lipids. Total lipids, chylomicrons, unesterified fatty acids, glycerol, triolein and radioactive fats have been studied in this manner, but none is a perfect test.

Vitamin A was employed by J. L. Beaumont and J. Lenegre (*Am. Heart J.* 58:163, 1959) to study the tolerance of fifty-four anginal patients to fat. The authors reported that vitamin A esters behave as a tracer of neutral fat during the first stages of its metabolism.

A neutral vitamin A in oil was given to the subjects as follows: 100,000 units at 9:00 a.m. on the first and second days, none on the third day, and 500,000 units on the fourth. Breakfast was fed immediately after the test dose. Blood was collected on the first and fourth days before the test dose and at 3, 6, 9, 12 and 24 hours thereafter. The normal ranges for the vitamin A tolerance test were established in a group of nineteen patients with cardiac disease but without atherosclerosis.

The fifty-four patients were divided into the six following groups: seven with primary hypercholesterolemia, eight with essential hyperlipemia, two with diabetes mellitus and hyperlipemia, one with myxedema, twenty-four with atypical and usually moderate hyperlipemia, and twelve with normal fasting blood lipids.

The vitamin A tolerance test gave normal values for patients with essential hypercholesterolemia, and normal to elevated values for those with normal fasting blood lipids. However, the test gave abnormally high values for the patients with essential hyperlipemia, and variable results for those subjects with atypical hyperlipemia.

The authors interpreted these data as indicating a group of different metabolic disorders which have in common a disturbance of lipid metabolism and coronary artery disease. The vitamin A tolerance test seemed to be a means of identifying those subjects who had a disturbance of the preliminary stages of lipid metabolism (absorption, transportation and storage). There was a notable lack of correlation with the serum cholesterol.

The vitamin A tolerance test would appear to be a useful device for further investigation of lipid metabolism, especially in groups of patients who have alterations in rates of absorption, transportation and storage of neutral fats.

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Prognosis of Diabetes Mellitus with Onset during Pregnancy

A Clinical Study of Seventy-one Cases

Lars Hagbard, M.D., and Alvar Svanborg, M.D., Gothenburg, Sweden

It is well known that pregnancy may have a diabetogenic effect. The onset of diabetes often occurs in connection with pregnancy, and in individuals with diabetes the insulin requirement usually increases during the latter part of pregnancy. Transitory forms of diabetes occurring in conjunction with pregnancy have been noted by many writers.[1-10]

Important questions, such as whether pregnancy introduces a risk of permanent deterioration of the condition of a patient with diabetes, or if pregnancy or repeated pregnancies increase the risk of the occurrence of permanent diabetes in symptom-free individuals with latent diabetes, or even in completely healthy individuals, are of great practical significance but cannot yet be regarded as finally answered.

In the present investigation we have attempted to explore these problems through a detailed study of the symptoms and course of the disease in seventy-one women in whom diabetes was diagnosed during pregnancy and in whom it was possibly induced by the pregnancy. The patients' physicians suspected diabetes at one of the three regular examinations during the pregnancy or the patients with more pronounced symptoms sought care from a physician or at a hospital. All suspected cases were hospitalized and the presence of true diabetes was confirmed. As far as we know, only isolated cases of this type subjected to observation over extended periods have been published heretofore. The seventy-one cases are the total number with diabetes developing during pregnancy who have been delivered at some of the larger hospitals in Sweden during 1948-54. The total number of pregnancies in these districts is not known for this period.

MATERIAL AND METHODS

Diagnostic criteria. (1) The patients presented subjective symptoms of diabetes, e.g., polydipsia, polyuria, loss of weight, ketonuria, and possibly acidosis. (2) Glycosuria occurred in all cases. The fasting blood sugar level determined by the Hagedorn-Jensen method on capillary blood in the majority of cases lay in repeated examinations about or above 200 mg./100 ml. blood. In cases presenting symptoms of diabetes and glycosuria, but with lower fasting blood sugar values, oral glucose tolerance tests were performed using 1 gm. of glucose per kilogram body weight. A maximal blood sugar increase to above 200 mg./100 ml. blood and an abnormal delay in the return of the blood sugar curve to normal level were regarded as prerequisites to the diagnosis of diabetes. (3) Symptoms of other endocrine diseases that affect the carbohydrate metabolism, e.g., thyrotoxicosis, Cushing's syndrome and acromegaly, were not present.

The time of onset of the diabetes has been considered to be the time at which the diagnosis was established, i.e., generally in immediate connection with the demonstration of subjective diabetes symptoms or the detection of glycosuria. The patients were routinely examined three times during the pregnancy, in the period from the second to the fourth months of pregnancy, at midterm and from four to six weeks before the estimated time of delivery. The patients in whom symptoms of diabetes were observed were subjected to more frequent examinations.

Follow-up examination of all patients was carried out after the end of the lactation period since, according to our own experiences, the carbohydrate loss and the instability of the metabolic state during the lactation may cause transient changes in the insulin requirement. In addition, the transitory cases have been checked annually.

RESULTS

The age distribution at the onset of the diabetes is shown in table 1.

The course of diabetes in thirty-seven of the seventy-one cases, or in 52 per cent, was transitory, i.e., after delivery and lactation all symptoms of diabetes disappeared; the blood sugar values returned to normal; the

From the Department of Gynecology and Obstetrics and from the Medical Clinic II, Sahlgrenska sjukhuset, Gothenburg, Sweden.

TABLE 1

Age and parity at the onset of diabetes

Series	Parity I	II	III	IV	V or more	Total		Per cent	
Transitory cases									
-20 years	—	—	—	—	—	—	15	—	40
20-29 "	8	6	1	—	—	15		40	
30-39 "	3	1	5	3	2	14		39	
40- "	—	1	3	2	2	8		21	
Total	11	8	9	5	4	37			
Per cent	30	22	24	13	11			100	
Permanent cases									
-20 years	2	—	—	—	—	2	24	6	71
20-29 "	13	5	3	—	1	22		65	
30-39 "	3	3	1	2	1	10		29	
40- "	—	—	—	—	—	—		—	
Total	18	8	4	2	2	34			
Per cent	53	23	12	6	6			100	
Grand total	29	16	13	7	6	71			
Per cent	41	23	18	10	8			100	

glycosuria disappeared, and in doubtful cases where glucose tolerance tests were performed, the results were completely normal. These cases have been designated as *transitory cases*. In the remaining thirty-four cases symptoms persisted after the termination of the lactation period and the patients required treatment with insulin. These cases have been designated *permanent cases*.

Onset of diabetes in relation to age and parity. In somewhat more than half the total material, 55 per cent, the diabetes was diagnosed before age thirty (table 1).

Comparison of the age at onset in transitory cases and permanent cases shows the existence of a significant difference ($p<0.001$). In 71 per cent of the patients in whom the diabetes became permanent after the termination of the pregnancy the onset was before the age of thirty years as opposed to 40 per cent in the transitory cases. Moreover, diabetes appeared in two of the permanent cases before the age of twenty years but among the transitory cases there was none under the age of twenty years. In none of the permanent cases did diabetes first appear after the age of forty years, while the onset was after age forty in eight of the women with transitory diabetes.

Table 1 also shows the parity of the women at the time diabetes was diagnosed. It may be seen that in 41 per cent of the present material the diabetes was diagnosed during the first pregnancy, in 64 per cent during the first or second pregnancy and in 82 per cent during one of the first three pregnancies. However, in no less than 18 per cent, the disease was not diagnosed until the fourth pregnancy or later. The latest onset of diabetes occurred in one patient during the eleventh pregnancy; in this case the disorder was transitory.

Statistical analysis of the onset of diabetes in relation to the parity shows considerable differences between the transitory and the permanent cases. In the transitory cases onset occurred during the first pregnancy in 30 per cent. In the rest of these cases the onset occurred with evenly declining frequency with rising parity (table 1). Among the permanent cases diabetes was diagnosed in 53 per cent during the first pregnancy and in 76 per cent during one of the first two. Accordingly, in the cases in which the diabetes proves to be permanent after the termination of the pregnancy and lactation, the onset occurs at lower parity and the difference in comparison with the transitory cases is highly significant ($p<0.001$). Unfortunately the limited size of the material does not permit elimination of the influence of age for determination of the significance of the parity alone.

Onset of diabetes in relation to the trimester of pregnancy. Table 2 gives the stage of pregnancy when the disease was diagnosed. It is evident that in more than 60 per cent of the patients the disease was first diagnosed during the third trimester. It is of interest to note that there is no greater accumulation of cases with a late onset of diabetes among the transitory cases than among the permanent, which might possibly have been expected. There is no statistically demonstrable difference with respect to the time of onset.

TABLE 2

Onset of diabetes in relation to the trimester of pregnancy

Parity	Transitory cases Trimester 1	2	3	Permanent cases Trimester 1	2	3	Total series Trimester 1	2	3
I	1	5	5	3	4	11	4	9	16
II	1	2	5	2	—	6	3	2	11
III	1	4	4	1	—	3	2	4	7
IV	—	—	5	—	—	2	—	—	7
V or more	2	—	2	1	—	1	3	—	3
Total	5	11	21	7	4	23	12	15	44
Per cent	14	35	51	21	12	67	17	21	62

Insulin requirements during and after pregnancy. In order to gain a conception of the degree of severity of the disease the daily maximal insulin requirement during pregnancy was investigated. The results are given in table 3, where the insulin requirements after the pregnancy and lactation are also shown.

Somewhat more than half the patients (thirty-nine cases) had such pronounced diabetes during the preg-

TABLE 3

Maximal daily insulin requirement

Time of diagnosis	No.	Insulin dosage during pregnancy (in units)				Insulin dosage after pregnancy			
		0	≦24	24-48	≧48	0	≦24	24-48	≧48
Transitory Cases									
1 trimester	5	4	1	—	—	5	—	—	—
2 trimester	11	6	3	—	2	11	—	—	—
3 trimester	21	9	6	—	6	21	—	—	—
Total	37	19	10	—	8	37	—	—	—
Per cent	—	51	27	—	22	100	—	—	—
Permanent Cases									
1 trimester	7	1	2	1	3	—	3	1	3
2 trimester	4	1	1	—	2	—	1	1	2
3 trimester	23*	11	5	2	5	—	8	7	6
Total	34	13	8	3	10	—	12	9	11
Per cent	—	38	24	9	29	—	—	—	—
Grand total	71	32	18	3	18	37	12	9	11
Per cent	—	46	25	4	25	52	18	14	16

*Two patients died during pregnancy.

nancy that insulin administration became necessary. In the remaining thirty-two cases no insulin was given. In some of these cases only dietary restrictions seemed indicated. In certain cases insulin should actually have been administered during the pregnancy, but the disease was diagnosed only in connection with the delivery and after delivery and symptoms disappeared spontaneously, wherefore insulin therapy was never instituted. The thirteen cases that proved after the termination of lactation to be permanent diabetes, but were not under insulin treatment during pregnancy, were accordingly all first diagnosed at the time of delivery, and the patients should have received insulin during pregnancy.

In eighteen of the thirty-nine insulin-treated cases the maximal daily insulin requirement did not exceed 24 I.U. In three cases between 24 and 48 I.U. was given. In eighteen cases the dose exceeded 48 I.U., a high incidence of diabetes with a large insulin requirement. Eight of these patients were admitted in coma or with incipient coma and were then temporarily placed on extremely high quantities of insulin. Four of the precoma or coma cases were transitory in course. Four other patients with a maximal daily insulin requirement in excess of 48 I.U. did not need insulin after delivery and the termination of lactation. In one of the patients the insulin requirement increased after the pregnancy. At the time of follow-up examination of the patients after the termination of the lactation period only eleven of the surviving sixty-nine mothers used 48 I.U. or more insulin, i.e., 16 per cent.

Comparison of the maximal daily insulin requirements in the transitory cases and in the cases that proved to have permanent diabetes (table 3) shows the existence of a highly significantly greater insulin requirement in the permanent cases ($p<0.001$). The difference is actually even more marked since the group of thirteen permanent cases, as pointed out earlier, really should have received insulin therapy during pregnancy.

Theoretically it should be possible from this to draw the conclusion that the less the insulin requirement the greater the chance will be that the diabetes will prove transitory. There are, however, as can be seen in table 3, a number of exceptions to this generalization. For instance, eight cases with maximal insulin requirements during pregnancy proved transitory, while twenty-one patients with insulin requirements not exceeding 24 I.U. proved to have permanent diabetes.

In the present material it is not possible to demonstrate any difference in the insulin requirements between cases presenting early onset during the pregnancy and cases presenting late, although this might possibly have been anticipated. It is also remarkable that the length of the period during the pregnancy, when insulin had to be administered, did not affect the insulin requirement after the pregnancy.

Diabetic coma and incipient coma. Ketosis of varying degrees of severity occurred in 40 per cent of the cases, and the incidence was somewhat higher in the permanent cases. The difference is not statistically significant, however. Eight patients were admitted to the hospital in coma or incipient coma comprising four permanent cases and four transitory. Thus, it is remarkable that four patients, who present such severe diabetic symptoms in connection with pregnancy, apparently recover completely postpartum with disappearance of all diabetic symptoms and signs. These four cases are reported in greater detail in another connection.[11]

Maternal mortality. Two mothers died in connection with pregnancy during which their diabetes was diagnosed. The cause of death in each case was diabetic coma. Neither of the patients knew of the disease and reached the hospital so late that it was impossible to save them despite intensive treatment.

Hereditary conditions. An analysis of the hereditary conditions in these seventy-one cases showed that 38 per cent of the patients had diabetic hereditary backgrounds within the circle of parents, brothers or sisters, and maternal or paternal grandparents. Comparison with a more extensive material with manifest diabetes in conjunction with pregnancy,[12] in which 40 per cent had a positive diabetic heredity, shows that no difference is

apparent. Nor could any statistically significant difference be demonstrated between transitory and permanent cases in this respect.

Follow-up study of transitory cases. Repeated annual checks were carried out in the thirty-seven cases in which the diabetes proved transitory following pregnancy during which it was diagnosed. The follow-up examinations were carried out by means of questionnaires sent to the patients in which they replied to questions concerning current disease symptoms, insulin requirement, pregnancies, etc. In cases where answers to these questions were in the affirmative, data were obtained from doctors treating the patients or from hospital records. Four women could not be found because of change of residence. Thus the follow-up investigation includes thirty-three women with transitory gestational diabetes. The results of this follow-up study will be reported in greater detail in another publication.[11] A survey of the present results is presented in table 4.

TABLE 4

The results of a follow-up study of thirty-three cases with transitory gestational diabetes

Follow-up			Transitory diabetes			Permanent diabetes		
				Pregnant				
Years	Number of cases	No symptoms	Infection	Insulin treated	No insulin	Infection	Pregnant	Unknown
1	33	28	—	1	3	1		
2	22	19	—	1			1	1
3	15	11	—		1		1	
4	11	11	—					
5	8	7	—				1	
6	6	4	—			1		1
7	4	1	—				2	1
Total			—	2	4	2	5	3

As may be seen, the length of the observation period varies widely. We have, even though the observation time was short in many cases, found ten patients with manifest insulin-treated diabetes, i.e., at least 27 per cent of the women with transitory diabetes of pregnancy finally developed diabetes. The manifest symptoms of the disease in five of these cases appeared in connection with a pregnancy, in two cases in association with severe infectious diseases and in three without any demonstrable cause.

Eleven of the thirty-three women subjected to follow-up examination again became pregnant during the observation period, one of the women twice. All presented symptoms of diabetes in conjunction with the pregnancy. In five cases the diabetes proved to be permanent after the delivery and lactation period; in six cases the disorder was again transitory.

Perinatal mortality. In the series with permanent diabetes the perinatal mortality was 38.2 per cent and in the series with transitory diabetes 43.2 per cent.

DISCUSSION

A transitory course of diabetes mellitus was observed in thirty-seven of the sixty-nine patients who survived pregnancy and delivery. If this observation were applicable more generally, accordingly, slightly more than half the patients with symptoms of diabetes observed first during pregnancy, would become symptom-free after the completion of the pregnancy and termination of the lactation period.

The diagnosis of diabetes was established in all cases in connection with pregnancy. Certain observations, however, indicate that some of the patients had had symptom-free (latent) diabetes even before the beginning of the pregnancy. The diagnosis was established in 17 per cent of the cases during the first trimester, i.e., during the part of the pregnancy when the insulin requirement in individuals with permanent insulin-treated diabetes is not usually increased. The establishment of the diagnosis at that time, at least in certain cases, seems to have been due to the fact that the patients were examined then because of their pregnancy and the diabetes discovered by chance. In six cases a period of abnormal thirst was reported as having occurred in conjunction with an earlier pregnancy, but sufficient criteria for establishing an earlier onset of the disease than at the time of the current pregnancy were not available. The perinatal fetal mortality before the time the diagnosis of diabetes was established was also remarkably high. Earlier, forty-seven of the seventy-one women had borne altogether ninety-eight children, with 22.5 per cent perinatal mortality.

Thus, these observations definitely indicate that many of these patients had mild diabetes without symptoms even before the pregnancy during which their disease was diagnosed. Milder forms of diabetes were formerly thought to occur principally in the higher age groups, especially over the age of forty-five years. In one of the first investigations carried out on a large population group Blotner[13] showed that mild, symptom-free cases of diabetes are common even among younger individuals. Since that time numerous investigators have shown that the number of symptom-free, undiagnosed cases of diabetes is approximately as great as the number of known cases.[14–16]

The importance of detecting and treating these mild cases of diabetes early has been strongly emphasized in recent years in the literature. In the present series the perinatal mortality was considerably higher than it would

have been if the disease had been diagnosed and adequately treated in time. Moreover, two of the pregnant women died in diabetic coma. Neither of the patients knew of the disease and reached the hospitals so late that it was impossible to save them despite intensive treatment. The possibilities of discovering the disease appear to be especially favorable during pregnancy, and it would therefore be desirable if a thorough check on pregnant women with respect to the existence of latent diabetes could be more generally instituted.

It would have been of great interest to know something about the possible role of obesity in the tendency toward the development of diabetes during or after pregnancy. Unfortunately we have insufficient data on this matter.

In order to determine if the prognosis of diabetes diagnosed in connection with pregnancy can be established during the pregnancy we made various comparisons between transitory and permanent cases. In the beginning it seemed probable to us that the patients who developed permanent diabetes after the pregnancy would have a more severe form of the disease than the patients in whom the course of the disease was transitory. However, our investigations do not show that such is the case.

In individuals with known diabetes even before pregnancy the insulin requirement usually rises only in the latter part of the pregnancy. We could therefore expect a greater number of cases with onset of diabetes at the end of the pregnancy in the present material, and such proved to be the case. During the third trimester 62 per cent of the cases were detected. In addition to these, however, it might have been expected that there would have been an earlier onset of diabetes in the cases in which permanent diabetes developed than in those that proved transitory. Statistical analysis of our material definitely shows, however, that such is not the case. Accordingly, there is no possibility of evaluating the prognosis after the completion of the pregnancy from the time of the onset of the disease during the pregnancy.

A pronounced difference in the magnitude of the insulin requirement in the permanent and transitory cases was observed. On the whole, therefore, the conclusion can be drawn that, the smaller the amount of insulin required during pregnancy is, the greater the chance will be that diabetes will prove transitory. However, there are a number of exceptions to this rule. Some cases with maximal insulin requirements during pregnancy in excess of 48 I.U. proved transitory, while other cases with insulin requirements not exceeding 24 I.U. developed into permanent diabetes. It is also remarkable that the length of the period during which insulin was administered during the pregnancy did not seem to affect the insulin requirement after the completion of the pregnancy. Nor do these observations indicate that adequate insulin substitution during pregnancy decreases the risk of permanent deterioration of the diabetic condition, a view advanced by Hoet and Brasseur[17] and Hoet and Lukens.[18]

The severe cases, indicated by the occurrence of ketonuria or acidosis, occurred almost equally in the two groups. It is remarkable that four of the six surviving patients with coma or incipient coma at the onset of the disease completely recovered after the end of the pregnancy and no longer presented either subjective or objective symptoms of diabetes. Similar cases have been described earlier.[9, 19]

The best bases for evaluation of the prognosis of the disease after the pregnancy are the age and parity at the onset of diabetes and the insulin requirement. It is evident from table 5, for example, that a woman under thirty years of age and pregnant for the first or second time has about a 40 per cent chance that the diabetes will prove transitory. If she is over thirty years of age and pregnant for the third time or more, the chance the disease will be transitory increases to about 70 per cent. The older she is and the higher her parity at the time the disease is diagnosed, the greater the possibility becomes that the disease symptoms will disappear completely postpartum at the termination of the lactation period.

TABLE 5

Age and parity in relation to the prognosis of the disease after pregnancy

		Transitory cases			Permanent cases		
Age	No.	No.	Per cent		No.	Per cent	
-20 years	2	—	—	38	2	100	62
20-29 "	37	15	40		22	60	
30-39 "	24	14	58	69	10	42	31
40- "	8	8	100		—	—	
Parity							
I	29	11	38	42	18	62	58
II	16	8	50		8	50	
III	13	9	69		4	31	
IV	7	5	71	69	2	29	31
V or more	6	4	67		2	33	

The practical, extremely important questions whether pregnancy gives rise to permanent aggravation of preexisting diabetes and whether or not pregnancy increases the tendency to permanent diabetes in individuals with latent diabetes or individuals with a predisposition to diabetes, cannot as yet be answered definitely. The increased production of diabetogenic hormones during

pregnancy subsides rapidly after delivery, wherefore the basis for permanent aggravation of the disease seems to be lacking. On the other hand, the possibility must be considered that a prolonged strain on the insulin-producing apparatus during pregnancy might cause permanent damage. This is negated by the observations in the present investigation since the duration of the disorder during pregnancy did not affect the prognosis of the disease after the pregnancy.

With respect to women with manifest diabetes even before the start of pregnancy the majority of writers have been unable to demonstrate a permanent increase in the insulin requirement after completion of pregnancy,[10, 12, 19–22] which should be the case if pregnancy has a tendency to cause permanent aggravation of the disease. The changes in the insulin requirement observed by the investigators cited include a decrease as often as an increase, and the order of magnitude of the changes is similar to variations in insulin requirements that may be observed even in individuals who have not undergone pregnancy.

Although it can be said with a high degree of probability that a woman with manifest diabetes generally need not anticipate permanent aggravation of her disease as a result of pregnancy, this does not necessarily mean that the same is true of patients with latent or mild forms of diabetes. In the literature we find considerable agreement that pregnancy can provoke manifest forms of diabetes in individuals with a tendency to diabetes. Diabetes is believed to be a recessive hereditary, not sex-bound, disease, which is, however, more common in women than in men of middle age.[23–25] Pregnancy is generally considered to be a contributing factor. Pyke,[25] who investigated 953 diabetic persons, concluded that "the excess of women found among diabetics is confined to those who have borne children and rises with increasing parity." Murphy[15] examined forty women who had each borne at least ten children and found that 54 per cent of these women had previously undetected diabetes. Hoet[26] found lower frequency of heredity among diabetic women who had undergone many pregnancies than among diabetic women who had not borne children or undergone pregnancies. His interpretation was that "presumably the physiologic derangements associated with pregnancy played an important part in the diabetogenesis among the former women." In our opinion one of the most important proofs of the diabetogenic effect of pregnancy is Katsch's[27] study on six pairs of enzygotic twins. He found that the sisters who underwent pregnancy earliest or who underwent the greater number of pregnancies developed diabetes earlier than their twin sisters. Moreover, this view is also supported by observations by many investigators that diabetes, in frequencies ranging from 14 to 44 per cent, manifests itself clinically for the first time in connection with pregnancy.[12]

It must be emphasized that if a person has ever shown an unmistakable abnormality in carbohydrate metabolism, that person must be considered as a (latent) diabetic. This is quite evident from the present study, since ten out of thirty-three patients, with transitory gestational diabetes, were reported in follow-up to have developed manifest diabetes (in five of them after another pregnancy). On the other hand, six women had undergone new pregnancies without the development of permanent diabetes.

The present investigation includes thirty-four women who developed manifest diabetes after pregnancy. Definite proof that this morbidity rate is higher than that among nonpregnant women during a corresponding period of time and of corresponding ages will not be available as long as apparently healthy individuals are not subjected to similar study. Thus, this investigation gives no definite proof that pregnancy increases the risk that latent forms of diabetes will become manifest.

Therefore it is difficult to say whether pregnancy is advisable for women in whom latent diabetes is suspected, considering the risk of permanent aggravation of the disease condition. There seems to be justification for saying, however, that, even if a certain risk of aggravation of the disease cannot be precluded, the results of the present investigation indicate that this risk is not great.

SUMMARY

In thirty-seven of seventy-one cases of diabetes with onset during pregnancy the course was transitory.

The age and parity at onset were significantly lower in the women in whom the disease proved to be permanent after the completion of pregnancy and termination of lactation than in the women with transitory diabetes.

The duration of the disease or the length of the period during which insulin had to be administered during pregnancy did not affect the insulin requirement after pregnancy.

The mean insulin requirement during pregnancy was found to be significantly higher in the cases of permanent diabetes but there are a number of exceptions to this rule.

Ketosis and diabetic precoma or coma were as common in transitory as in permanent diabetes.

It appears that the age and parity of the women at

onset and the insulin requirement should be considered primarily in prognostic evaluation of the continued course of diabetes developed during pregnancy.

In follow-up studies of thirty-three of the thirty-seven women with transitory gestational diabetes up to seven years postpartum, ten were found to have developed manifest diabetes, requiring insulin treatment.

The questions are discussed whether pregnancy is accompanied by the risk of creating permanent aggravation of diabetes, or increases the risk of the occurrence of manifest diabetes in individuals with latent diabetes.

SUMMARIO IN INTERLINGUA

Le Prognose de Diabete Mellite in Casos con Declaration Durante le Pregnantia: Un Studio Clinic de Septanta-Un Casos

In trenta-septe inter septanta-un casos de diabete con declaration durante le pregnantia del patiente le curso del morbo esseva transitori.

Le etate e le frequentia de previe parturitiones esseva significativemente plus basse in le feminas in qui le morbo permaneva post le completion del pregnantia e le termination del lactar que in le feminas in qui le diabete esseva transitori.

Le duration del morbo o le longor del periodo quando insulina debeva esser administrate durante le pregnantia non afficeva le requirimentos de insulina post le completion del pregnantia.

Esseva constatate que le requirimento medie de insulina durante le pregnantia esseva significativemente plus alte in le casos de diabete permanente que in le casos de diabete transitori. Tamen, in su application a casos individual, iste regula revelava un numero de exceptiones.

Cetosis o precoma o coma diabetic esseva tanto commun in diabete transitori como in diabete permanente.

Il pare que le etate e le numero del previe parturitiones del feminas al tempore del declaration de lor diabete e etiam lor requirimentos de insulina debe esser considerate primarimente in le evalutation prognostic del curso futur de diabete que se ha declarate originalmente durante un pregnantia del patiente.

In studios sequential de trenta-tres del trenta-septe feminas con transiente diabete gestational effectuate a periodos de usque a septe annos post parto, il esseva trovate que dece habeva disveloppate diabete manifeste que requireva tractamento a insulina.

Es discutite le questiones de si le evenimento del pregnantia es accompaniate del risco de un causation de permanente aggravation de diabete o si le evenimento del pregnantia augmenta le risco del occurrentia de diabete manifeste in individuos con diabete latente.

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Sickness and Injury Experience of Employed Diabetics

Sidney Pell, Ph.D., and C. A. D'Alonzo, M.D., Wilmington

In recommending standards for the employment of diabetics, the Committee on Employment of the American Diabetes Association[1] has expressed the view that "controlled diabetics without complications are good employable risks." The Committee also states: "When it is said that a diabetic is a good employment risk, reference is made to the well-controlled, well-regulated diabetic who keeps himself constantly under medical supervision. These people should prove to be quite acceptable, as the experience of hundreds of companies employing such diabetics indicates. Indeed, because they are self-disciplined and well-balanced individuals, they often become outstanding workers in whatever positions they occupy." Soskin,[2] citing his personal experience with the diabetic employee, believes that "the feeling engendered by the nonprejudicial acceptance of his handicap is apt to make him an unusually hard-working and loyal employee." Beardwood[3] surveyed thirty-one industrial firms in the Philadelphia area, and reported instances where work performance and attendance of diabetics compared favorably with those of nondiabetics. He also uncovered prejudices against diabetics which resulted from unfavorable isolated experiences and ignorance of the nature of the disease on the part of employers. Brandaleone and Friedman,[4] studying forty diabetics in the Third Avenue Transit System in New York City, found that, apart from two diabetics who had very prolonged absences, the absence rate of the diabetics was about the same as that of the nondiabetics. They also found no difference in accident rates and a somewhat lower rate of nonmedical absences among the diabetics. In reporting the experience of ninety-two diabetics in the Home Office of the Metropolitan Life Insurance Company, Dublin and Marks[5] cited the favorable record of these diabetics from the standpoint of length of service, attendance and cooperation with company physicians. They believe that their study and others which they reviewed "indicate that the work record of diabetics in industry is satisfactory."

In general, these observations and comments express a plea to employers to take into account the distinction between the well-controlled diabetic and the poorly controlled, uncooperative diabetic; to assess each prospective diabetic employee on an individual basis; and to understand the relation of the diabetic's condition to job requirements.

As sound as these views are, there appears to be a reluctance on the part of a large number of employers to hire diabetics. In a recent survey of business and industrial firms conducted by the Committee on Employment of the American Diabetes Association,[6] about one quarter of the 127 participating companies stated that they do not employ known diabetics. The most frequently stated reasons were "previous poor experience" and "insurance reasons."

Before adopting a policy with respect to the hiring of diabetics, the employer has a right to know what he can expect from diabetics in such matters as attendance, frequency of disabling illness and safety performance. Although many employers have expressed favorable opinions in this respect, there is a dearth of statistical information on the subject. To our knowledge, the only well-controlled study in this country was done by the then Department of Labor from 1945 through 1947 as part of an industrial survey to evaluate the performance of physically impaired workers.[7]

As a means of appraising some aspects of the diabetic as an employee, we undertook a survey of all known diabetics in the du Pont Company. In this article, we will report our findings on sickness absenteeism, injuries, and status of diabetes control. Information concerning productivity and work efficiency lies outside the scope of our research activities.

In addition to gathering data on the items just cited, our survey enabled us to obtain prevalence figures and a great deal of laboratory and clinical information with which to study factors that have a bearing on the etiology and course of the disease. These findings are presented in another paper.[8]

The authors are Biostatistician and Assistant Medical Director, respectively, Medical Division, E. I. du Pont de Nemours and Co., Inc., Wilmington, Delaware.

SOURCE OF MATERIAL AND SURVEY PROCEDURES

Company medical facilities, policies, and records

During the past twenty-five years, it has been the practice of the du Pont Company to provide periodic physical examinations to all employees. These examinations are given, in almost all instances, by Company physicians. The laboratory work includes hemoglobin determinations, white blood cell counts, and urinalyses to test for the presence of sugar and albumin. More intensive blood and urine studies are performed when indicated. The routine examination also includes a chest X ray and, for older employees, an electrocardiogram.

Almost all employees have been receiving their medical examinations once a year, but many are seen more frequently for consultations and special examinations to detect possible adverse effects of potential occupational hazards. In addition, production employees who are absent for reason of illness or injury must report to the plant medical unit for evaluation upon their return to work.

When an employee exhibits abnormal conditions that require treatment or further diagnostic measures, he is referred to his family physician or a specialist. Contact between the Company and the local medical community is maintained to follow up cases that arise. This contact is also needed in the administration of the Company sickness benefit plans, which require the attending physician to certify the cause of disability.

Employees with glycosuria are given postprandial blood sugar or glucose tolerance tests. Most Company installations have facilities to administer these tests, but those which do not, have the work done at a local laboratory. Some Company physicians have blood sugar determinations made when there is a family history of diabetes without glycosuria. All employees suspected of having diabetes are followed closely until a diagnosis of diabetes is clearly established or rejected.

The Company does not refuse employment to anyone because of diabetes mellitus, except when the applicant cannot demonstrate that he is able to control the disease. The suggested policy and procedure for Company physicians is stated as follows: "Uncontrolled diabetes is a severe hazard. These cases should not be accepted, under any circumstances, until the blood sugar is within normal limits and medically controlled. If the applicant is accepted, he should be followed up every three months or so, or at least until the examiner satisfies himself that the diabetes is well managed."[9] Diabetic employees are encouraged to seek the help and advice of their plant physicians for problems concerning the management of their diabetes.

Survey procedures

Our survey was begun in June, 1956. At that time, we distributed questionnaires, dealing with various aspects of diabetes, to all Company physicians, and asked them to complete the questionnaire by interviewing every diabetic in their plant. Thus, the Company physician was given the responsibility of identifying the diabetics. Since each doctor is assigned about 800 employees, the names of the few diabetics seen by them could easily be recalled. Nevertheless, we allowed a little more than a year to elapse for the completion of the questionnaires. During that time, each physician examined all of his patients at least once, and was in a position to identify diabetics not recalled by him at the start of the survey.

The choice of diagnostic criteria was left, of course, to the judgment of the employee's personal physician. In questionable cases, we recommended to the Company physician that, for purposes of this study, a diagnosis of diabetes be made when the blood sugar exceeds 170 mg. per 100 ml. in one half to one hour after the ingestion of a test meal (or a meal high in carbohydrates) and/or when the blood sugar does not return to a level below 150 mg. per 100 ml. two hours after the meal.

After obtaining the names of all the known diabetics in the Company, we selected a control employee, matched to each diabetic by age (five-year categories), sex, occupation (i.e., production or salaried)* and location. The controls were selected by means of random numbers from a complete listing of Company employees.

We then requested the medical records of the diabetics and controls to abstract information pertinent to our study. We also asked the medical or personnel units in each installation to list for us all of the sickness absences and injuries that were experienced by the diabetics and controls during the year 1956, including the cause of illness and number of days of disability.

PREVALENCE OF DIABETES

During the one-year period in which the survey was conducted, Company physicians reported 408 cases of diabetes among 90,596 surveyed employees, a prevalence rate of 4.5 per 1,000 persons. These cases comprise 370 men and 38 women. Prevalence rates, shown in table 1, indicate the well-known increase in prevalence with age. In our figures, the sharpest increase

*Production employees include craftsmen, operatives, service workers, and laborers. Salaried employees consist of professional, technical, clerical, administrative, and managerial personnel.

TABLE 1
Prevalence of diabetes mellitus by age and sex
(Rates per 1,000)

Age	All employees		Men		Women	
	No.	Rate	No.	Rate	No.	Rate
Under 20	1	1.3	1	2.8	0	0
20-24	1	0.1	0	0	1	0.4
25-29	8	0.6	8	0.7	0	0
30-34	29	1.9	26	2.0	3	1.3
35-39	50	3.4	47	3.7	3	1.5
40-44	56	4.3	52	4.6	4	2.7
45-49	54	5.1	50	5.3	4	3.1
50-54	76	10.2	68	10.3	8	9.4
55-59	68	14.7	60	14.5	8	15.7
60-64	65	21.3	58	21.0	7	23.8
Total	408	4.5	370	4.8	38	2.7

occurs at about fifty years.

The over-all prevalence rate is higher among men than women; i.e., 4.8 per 1,000 compared to 2.7 per 1,000. This difference, however, is due, to some extent, to the higher proportion of older persons among the male employees. When the rates are standardized for age, the respective prevalence rates for men and women are 4.7 per 1,000 and 3.4 per 1,000.*

The prevalence rates in the Company should not be projected to the general population because of the select nature of the Company employees. This point is discussed in greater detail in the article previously cited.[8]

SICKNESS ABSENTEEISM

Company medical units and personnel sections provided us with a complete record of sickness absenteeism during the year 1956 for 389 of the 408 diabetics and 382 of the 408 nondiabetic controls. The records sent to us indicated the cause of illness and the number of days of disability for each absence.

Differences between diabetics and controls

Comparing the two groups as a whole, the diabetics experienced a somewhat higher rate of sickness absenteeism than did the nondiabetics, from the standpoint of both frequency and severity. There were a total of 220 absences among the 389 diabetics and 181 among the 382 controls. Expressing these figures as frequency rates, we obtain, respectively, 56.6 and 47.4 per 100 persons. The probability of this difference occurring by chance alone is less than 0.01.

Severity indices were also higher in the diabetic group. The mean number of days of disability among diabetics with one or more absences was 28.0; among the controls, 17.5. The median number of days was 12.6 in the diabetic group and 7.0 in the control group. The difference between the two medians is expected to occur by chance alone less than one time in twenty.

*The rates were standardized by the indirect method, using the over-all Company rates as the standard rates.

The 389 diabetics were disabled for a total of 4,200 days, an average of 10.8 days per person, while the 382 controls were disabled for a total of 2,150 days, an average of 5.6 days per person.

Eighty-six of the 389 diabetics, 22.1 per cent, were disabled during the year for a total of ten or more days. This is compared to 13.7 per cent among the controls ($P<.01$). Disability of thirty or more days occurred in 9.7 per cent of the diabetics and 5.8 per cent of the controls ($P=.05$).

Cumulative frequency distributions of the number of sickness absences and the number of days of disability among the diabetics and controls are shown in tables 2 and 3. The curves shown in figure 1 indicate that, from the time of onset of disability, diabetics return to work at a somewhat slower rate than do the nondiabetics.

In table 2 it is worth while noting that 86.4 per cent of the diabetics had either no absences or only one absence during the year, and only 4.1 per cent were absent more than twice, indicating that the problem of absenteeism among the diabetics, as well as nondiabetics, is concentrated in a small minority of individuals.

Variation by age, occupation, and severity of diabetes

Although sickness absenteeism among the diabetics as a whole was greater than that of the controls, differences between the two groups varied according to age, occupation, and severity of diabetes. The effect of these factors is seen in table 4 where several indices of sickness absenteeism are presented. In making comparisons with the controls, the three variables were analyzed by the following dichotomous subdivisions: Age, under fifty years and fifty to sixty-four years; occupation, production employees and salaried employees; and se-

TABLE 2
Number of sickness absences during 1956 among diabetics and controls

Number of sickness absences	Diabetics		Controls	
	No.	Per cent	No.	Per cent
None	240	61.7	259	67.8
One	96	24.7	92	24.1
Two	37	9.5	18	4.7
Three	14	3.6	7	1.8
Four	2	0.5	3	0.8
Five or more	0	0	3	0.8
Total no. of cases	389	100.0	382	100.0

Cumulative frequency distribution:

Number of sickness absences	Diabetics		Controls	
	No.	Per cent	No.	Per cent
One or more	149	38.3	123	32.2
Two or more	53	13.6	31	8.1
Three or more	16	4.1	13	3.4
Four or more	2	0.5	6	1.6
Five or more	0	0	3	0.8

TABLE 3
Number of days of disability during 1956 among diabetics and controls

Number of days of disability	Diabetics		Controls	
	No.	Per cent	No.	Per cent
None	240	61.7	259	67.8
1 to 4	34	8.7	48	12.5
5 to 9	29	7.5	23	6.0
10 to 14	22	5.7	9	2.4
15 to 19	10	2.6	8	2.1
20 to 29	16	4.1	13	3.4
30 to 59	20	5.1	13	3.4
60 to 89	7	1.8	6	1.6
90 or more	11	2.8	3	0.8
Total no. of cases*	389	100.0	382	100.0

Cumulative frequency distribution:

Number of days of disability	Diabetics		Controls	
	No.	Per cent	No.	Per cent
1 or more	149	38.3	123	32.2
5 or more	115	29.6	75	19.7
10 or more	86	22.1	52	13.7
15 or more	64	16.4	43	11.3
20 or more	54	13.8	35	9.2
30 or more	38	9.7	22	5.8
60 or more	18	4.6	9	2.4
90 or more	11	2.8	3	0.8

*Does not include nineteen diabetics and twenty-six controls for whom absence records were not available.

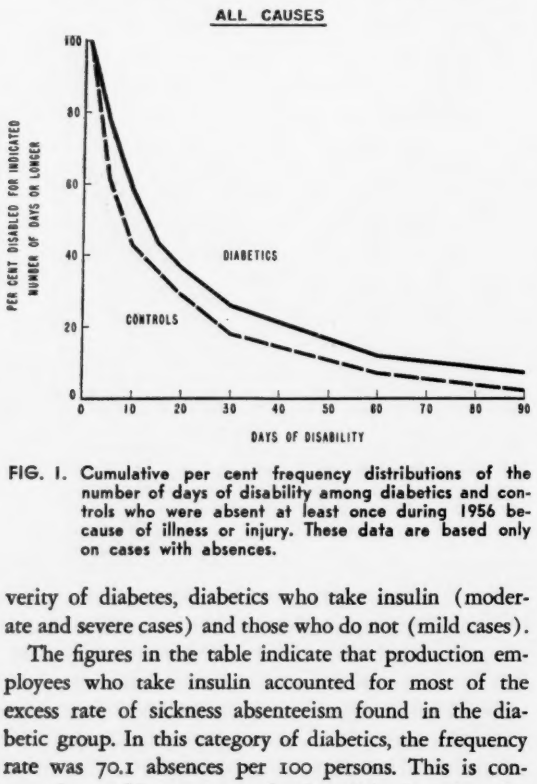

FIG. 1. Cumulative per cent frequency distributions of the number of days of disability among diabetics and controls who were absent at least once during 1956 because of illness or injury. These data are based only on cases with absences.

verity of diabetes, diabetics who take insulin (moderate and severe cases) and those who do not (mild cases).

The figures in the table indicate that production employees who take insulin accounted for most of the excess rate of sickness absenteeism found in the diabetic group. In this category of diabetics, the frequency rate was 70.1 absences per 100 persons. This is contrasted to 46.8 among production diabetics who take no insulin and 41.3 among the production employees in the control group (see figure 2). The probability of these differences occurring by chance alone is less than 0.01. Although the rate among the mild diabetics is somewhat greater than it is in the control group, the difference is not significant. A similar pattern is seen in rates of prolonged sickness absenteeism; i.e., the percentages of persons disabled for 10, 30, and 60 or more days. Among salaried employees, there were no significant differences in absence rates among the two diabetic groups and the control group.

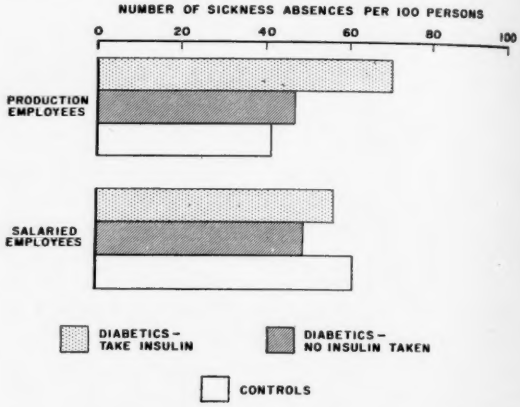

FIG. 2. Disability rates for all causes of illness or injury incurred during 1956 among diabetics and controls, by occupation and severity of diabetes.

The severity of the diabetes did exert some influence on the frequency of sickness absenteeism, but its effect was most pronounced in cases of very prolonged absences among the older production employees. Referring to table 4, it is seen that among production diabetics fifty to sixty-four years of age who take insulin the per cent disabled for thirty or more days was 19.2, compared to 10.3 among the mild diabetics and 5.4 among the controls ($P<.05$). The per cent disabled for sixty or more days in each of these groups was, respectively, 15.4, 3.8, and 3.1 ($P<.01$).

Causes of sickness absenteeism

A breakdown, by cause of illness, of the sickness absences that occurred in the diabetic and control groups during 1956 is shown in table 5. About one half of the absences in each group was due to respiratory infections and diseases of the digestive system. In neither of these disease categories is there a significant difference in incidence between the diabetics and nondiabetics. The absence frequencies for other illnesses are small, and none of the differences is greater than would be

TABLE 4

Sickness absenteeism during 1956 among diabetics and controls by age, occupation and severity of diabetes

	Severity	Both			Production			Salaried		
		All ages	Under 50 yrs.	50 to 64 yrs.	All ages	Under 50 yrs.	50 to 64 yrs.	All ages	Under 50 yrs.	50 to 64 yrs.
Number of persons	Diabetics—take insulin	200	118	82	127	75	52	73	43	30
	Diabetics—no insulin	189	73	116	126	48	78	63	25	38
	Controls	382	194	188	264	135	129	118	59	59
Number of absences per 100 persons	Diabetics—take insulin	65.0	72.0	54.9	70.1	82.7	51.9	56.2	53.5	60.0
	Diabetics—no insulin	47.6	45.2	49.1	46.8	47.9	46.2	49.2	40.0	55.3
	Controls	47.4 †	56.7 †	37.8 *	41.3 †	54.8 †	27.1 †	61.0	61.0	61.0
Per cent absent one or more times	Diabetics—take insulin	41.5	44.1	39.0	46.5	53.3	36.5	34.3	27.9	43.3
	Diabetics—no insulin	34.4	30.1	37.1	31.7	29.2	33.3	39.7	32.0	44.7
	Controls	32.2	36.6	27.7	29.2 †	35.6 †	22.5	39.0	39.0	39.0
Per cent disabled for 10 or more days	Diabetics—take insulin	26.0	26.3	25.6	33.9	36.0	30.8	12.3	9.3	16.7
	Diabetics—no insulin	18.0	16.4	19.0	19.0	16.7	20.5	15.9	16.0	15.8
	Controls	13.6 †	13.4 *	13.8	14.0 †	14.8 †	13.2 *	12.7	10.2	15.3
Per cent disabled for 30 or more days	Diabetics—take insulin	11.5	8.5	17.1	14.2	10.7	19.2	8.2	4.7	13.3
	Diabetics—no insulin	7.4	5.5	8.6	7.9	4.2	10.3	6.3	8.0	5.3
	Controls	5.8	6.2	5.3 †	5.7 *	5.9	5.4 *	5.9	6.8	5.1
Per cent disabled for 60 or more days	Diabetics—take insulin	7.0	4.2	11.0	9.4	5.3	15.4	2.7	2.3	3.3
	Diabetics—no insulin	2.1	1.4	2.6	2.4	0	3.8	1.6	4.0	0
	Controls	2.4 †	2.1	2.7 †	3.0 †	3.0	3.1 †	0.8	0	1.7
Median no. days of disability among persons absent one or more times	Diabetics—take insulin	14.0	13.0	16.0	15.0	14.3	35.0	5.5	5.5	5.8
	Diabetics—no insulin	10.7	11.5	9.5	15.0	11.0	15.0	7.3	11.0	6.0
	Controls	7.0 *	5.4 *	11.2	9.5	6.5	15.0	5.6	3.5	6.0

*The probability that the differences among the three groups will occur by chance alone is less than .05.
†The probability that the differences among the three groups will occur by chance alone is less than .01.

expected by chance alone. The higher over-all frequency of sickness absenteeism among the diabetics was due, primarily, to absences which resulted directly from their diabetic condition. In table 5, these absences are shown in the category, "Care and management of diabetes." There were twenty-five such absences, which occurred in twenty-three diabetics. Four of seven absences classified as "drug reactions" were due to insulin shock.

The incidence of absences due to diabetes is presented in table 6 by age, occupation, and severity of diabetes. These figures clearly show that these absences occurred predominantly among production employees under fifty years of age who take insulin. The incidence in this group was 17.3 per cent; i.e., thirteen absences among seventy-five diabetics.

As one would expect, absences due to diabetes were more frequent among the moderate and severe cases than in the mild group. These absences occurred in only 2.1 per cent of the diabetics who do not take insulin, but in 9.5 per cent of those who do take insulin ($P<.01$).

Duration of disability by cause

Table 7 shows the mean number of days of disability among the diabetics and the controls for respiratory infections, nonrespiratory illnesses, and diabetes. It can be seen that the duration of disability is greater among the diabetics than the nondiabetics for both respiratory and nonrespiratory illnesses.

Figure 3 shows cumulative distribution curves of duration of disability due to respiratory infections among the diabetics and the controls. The chart clearly shows that, after the onset of disability, the nondiabetics returned to work at a faster rate than did the diabetics. The median number of days of disability among the diabetics was 3.5, compared to 2.5 in the control group. Ten per cent of the controls and 25.4 per cent of the diabetics were disabled for one week or longer ($P<.05$).

DISPENSARY VISITS

Company medical units are equipped to provide first aid and medication to employees who report to the medical section with injuries and minor complaints.

The most frequently recorded reasons for coming to the dispensary are abrasions and lacerations, colds, simple headaches, gastrointestinal upsets, muscular pains, dysmenorrhea and allergies.

A comparison was made between the diabetics and controls with respect to the frequency of their dispensary visits during the two-year period, 1955-56. The findings, presented in table 8, show no significant differences between the two groups.

TABLE 5
Causes of sickness absenteeism

Cause of absence	Number of absences Diabetics	Controls
Respiratory infections	67	60
Diseases of digestive system	32	33
Musculoskeletal disorders	15	16
Nonrespiratory infections	13	13
Heart disease	8	3
Other cardiovascular disease	6	5
Drug reactions	7*	2
Genitourinary disorders	6	6
Accidents	5	3
Miscellaneous disorders	36	40
Care and management of diabetes	25	—
Total	220	181

*Includes four cases of insulin shock.

TABLE 6
Incidence of absence from work during 1956 for care and management of diabetes, by age, occupation and severity of diabetes

	Total	Number absent	Per cent absent
All diabetics	389	23	5.9
No insulin taken	189	4	2.1
Take insulin	200	19	9.5
Production:			
No insulin—under 50	48	2	4.2
No insulin—50 to 64	78	1	1.3
Takes insulin—under 50	75	13	17.3
Takes insulin—50 to 64	52	4	7.7
Salaried:			
No insulin—under 50	25	0	0
No insulin—50 to 64	38	1	2.6
Takes insulin—under 50	43	2	4.7
Takes insulin—50 to 64	30	0	0

ACCIDENT RECORD

In studying accident susceptibility we find that the occurrence of minor (nondisabling) injuries yields much more information than major and submajor injuries. Apart from the much greater frequency of minor injuries, the differences between the occurrence of a major and a minor injury are dependent upon chance and the nature of the external situation, rather than the degree of accident susceptibility of the person or persons involved.

Records of minor injuries were available for 384 diabetics and 382 controls. During 1956, the diabetic group experienced eighty-two injuries, or 21.4 per 100 persons. In the control group there were seventy-eight injuries, a rate of 20.4 per 100. The frequency distributions, in table 9, are almost the same for both groups, and the difference can be ascribed to chance.

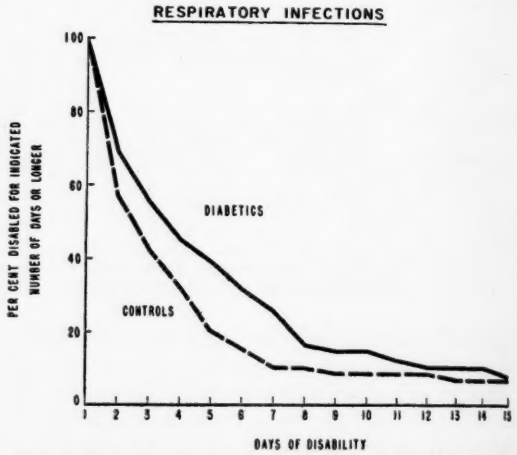

FIG. 3. Cumulative per cent frequency distributions of the number of days of disability among diabetics and controls who were absent at least once during 1956 because of respiratory infections. These data are based only on cases with absences.

TABLE 7
Duration of disability among diabetics and controls by type of illness

Type of illness	Diabetics Number of absences a	Total number of days of disability b	Mean number of days of disability Per absence b/a	Per person b/389	Controls Number of absences a	Total number of days of disability b	Mean number of days of disability Per absence b/a	Per person b/382
Respiratory infections	67	358	5.3	0.9	60	222	3.8	0.6
Nonrespiratory illnesses	124	3,407	27.5	8.8	121	1,928	15.9	5.1
Diabetes	29	435	15.0	1.1	—	—	—	—
All absences	220	4,200	19.1	10.8	181	2,150	11.9	5.6

It should be borne in mind that records of minor injuries list only those which were reported by the worker. In some instances, the injured person may not believe his injury to be severe enough to require medical attention. Diabetics, however, are generally aware that their wounds, no matter how slight, may become seriously infected if not given prompt attention. For this reason, a diabetic is more likely to report an injury than a nondiabetic. If this is true, the rate of minor injuries may actually be lower in the diabetics.

TABLE 8

Dispensary visits, exclusive of retreatments, among diabetics and controls, 1955-56

Number of dispensary visits	Diabetics		Controls	
	No.	Per cent	No.	Per cent
None	33	13.4	43	16.4
1 or 2	70	28.4	68	25.9
3 or 4	54	22.0	51	19.5
5 or 6	34	13.8	35	13.4
7 or 8	24	9.8	28	10.7
9 or 10	9	3.7	15	5.7
11 or 12	12	4.9	6	2.3
13 or 14	6	2.4	5	1.9
15 or more	4	1.6	11	4.2
Total no. of cases*	246	100.0	262	100.0

Cumulative frequency distribution:

Number of dispensary visits	Diabetics		Controls	
	No.	Per cent	No.	Per cent
1 or more	213	86.6	219	83.6
3 or more	143	58.2	151	57.7
5 or more	89	36.2	100	38.2
7 or more	55	22.4	65	24.8
9 or more	31	12.6	37	14.1
11 or more	22	8.9	22	8.4
13 or more	10	4.0	16	6.1
15 or more	4	1.6	11	4.2

*Records of dispensary visits were not maintained for 162 diabetics and 146 controls. Most of these employees are salaried.

With regard to other types of injuries that occurred during 1956, there were two major injuries among the diabetics and none in the control group. Both groups experienced three submajor injuries. Five diabetics and three controls suffered lost-time nonoccupational injuries. All of these differences are within the limits of sampling error.

The occurrence of injuries and sickness absenteeism among diabetics and nondiabetics was studied by the Bureau of Labor Statistics from 1945 through early 1947 in a sample of industrial firms.[7] Records of 144 diabetics were compared to those of 244 matched controls. All were production workers. Days lost because of sickness occurred at a higher rate in the diabetic group; i.e., 2.6 compared to 1.3 per 100 work days. Differences in injury rates were slightly higher among the diabetics, but it is questionable whether they are significant. The rate of nondisabling injuries, expressed as the number of injuries per 10,000 exposure hours, was 7.8 in the diabetic group and 7.4 in the control group. Disabling injuries occurred at a rate of 15.6 per 1,000,000 exposure hours among the diabetics and 12.9 among the controls.

TABLE 9

Minor injuries during 1956 among diabetics and controls

Number of injuries	Diabetics		Controls	
	No.	Per cent	No.	Per cent
None	332	86.5	327	85.6
1	36	9.4	38	9.9
2	8	2.1	12	3.1
3	4	1.0	4	1.1
4	2	0.5	1	0.3
5	2	0.5	0	0
Total	384	100.0	382	100.0

Although different indices are used, the findings of our survey and that of the Bureau of Labor Statistics are essentially the same, insofar as they suggest that diabetic production workers experience a higher rate of sickness absenteeism than nondiabetics, but do not have a greater susceptibility to accidents.

DISCUSSION

In assessing the sickness absence experience of diabetics on the basis of the data just presented, these points should be stressed. First, of the thirty-nine excess absences in the diabetic group (220-181), twenty-five were classified under the heading of "Care and management of diabetes," and an additional four were due to insulin shock. Thus, twenty-nine of the thirty-nine excess absences, about 75 per cent, were due directly to the diabetic condition, rather than to complicating illnesses. Secondly, these twenty-nine absences occurred in twenty-seven persons. This is only 7 per cent of the entire group of diabetics. Thirdly, the excess absenteeism among the diabetics was concentrated primarily among moderate and severe production diabetics. Generally, absence rates of salaried diabetics and of mild diabetics in production work were about the same as those of nondiabetics. Fourthly, the large majority of diabetics do not pose a serious problem with regard to absenteeism. During a one-year period, 86.4 per cent of the diabetics had either no absences or only one absence and 95.9 per cent had two absences or less.

From these findings the conclusion may be drawn that rates of sickness absenteeism among diabetics as a whole might be reduced, to a large extent, to that of

nondiabetics by directing efforts toward obtaining better control in a small group of diabetics, particularly moderate and severe cases among production workers.

With regard to the duration of sickness absenteeism, there are two possible explanations for the excess noted among the diabetics. One is that the diabetes itself may contribute to the severity of other illnesses. Another, but more likely, explanation is that the family physician may treat the diabetics more conservatively than he would a nondiabetic for the same condition. For example, when the insulin dosage is increased due to fever, the family physician may believe that a few extra days at home are necessary to regulate insulin dosage to preclude insulin shock or glycosuria.

SUMMARY

A survey of diabetics in the du Pont Company uncovered 408 cases, a prevalence rate of 4.5 per 1,000 persons. To evaluate the sickness absence and injury experience of these diabetics, a control group was drawn at random from the Company population so that one control employee was matched to each diabetic by age, sex, occupation (i.e., production or salaried) and geographical location.

Rates of sickness absenteeism were greater among diabetics than nondiabetics from the standpoint of both frequency and severity, but the excess occurred primarily among mild and severe diabetic production workers. Absence rates among salaried diabetics and among mild diabetic production workers were about the same as those of the controls. Most of the excess absences in the diabetic group were due directly to their diabetic condition, rather than to complicating illnesses. During a one-year period, 86.4 per cent of the diabetics had either no absences or only one absence.

The rate of return to work following the onset of illness was somewhat slower in the diabetic group than in the control group. This was true for illnesses in general and for respiratory infections in particular.

There was no significant difference in the occurrence of minor injuries between the diabetics and the controls.

In general, the study indicates that the great majority of diabetics do not pose any special problem with regard to sickness absenteeism, and that diabetics are no more susceptible to injuries than are nondiabetics.

SUMMARIO IN INTERLINGUA

Maladia e Vulnerationes in Empleatos Diabetic

Un enquete al Compania du Pont constatava 408 casos de diabete, i.e. un incidentia de 4,5 per mille. Pro evalutar le absentismo pro maladia e le frequentia de vulnerationes in iste gruppo, un selection aleatori esseva effectuate in le population del compania de maniera que in le resultante gruppo de controlo omne subjecto esseva appareate con un subjecto del gruppo diabetic tanto in etate e sexo como etiam in typo de occupation (i.e. production o empleo salariate) e loco geographic.

Le incidentia de absentismo pro maladia esseva plus alte in diabeticos que in non-diabeticos, tanto ab le puncto de vista del frequentia como etiam ab le puncto de vista del severitate; sed le excesso occurreva primarimente in subjectos con moderate e sever diabete inter le travaliatores de production. Le absentismo inter le diabeticos salariate e inter le travaliatores de production con leve diabete esseva plus o minus le mesme como in le gruppo de controlo. Le plus grande parte del excesso in le absentias del gruppo diabetic esseva causate directemente per le condition diabetic plus tosto que per complicationes secundari. Durante un periodo de un anno, 86,4 pro cento del diabeticos habeva nulle absentia o solmente un.

Le retorno al travalio post le declaration del morbo esseva un pauco plus lente in le gruppo diabetic que in le gruppo de controlo. Isto valeva pro morbos in general e pro infectiones respiratori in particular.

Nulle differentia significative esseva constatate inter le diabeticos e le gruppo de controlo con respecto al occurrentia de vulnerationes minor.

A generalmente parlar, le studio indica que le grande majoritate del diabeticos non pone problemas special con respecto al absentismo pro maladia e que diabeticos non es plus susceptibile de contraher vulnerationes que non-diabeticos.

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Effect of High Blood Sugar Levels upon Splanchnic Sugar Output in Experimental Diabetes

B. Issekutz, Jr., M.D., G. Hetenyi, Jr., M.D., M. Forbath, M.D., H. Drischel, M.D., and A. Konig, M.D., Szeged, Hungary, and Greifswald, Germany

It is known that the elevation of the blood sugar concentration by means of glucose infusion increases the sugar uptake of the peripheral tissues not only in normal but also in pancreatectomized dogs. This was described by Soskin and Levine in 1937 on the basis of their balance studies carried out on eviscerated diabetic dogs.[1] Also in situ measurements (blood flow multiplied by arteriovenous difference) showed that the artificially induced hyperglycemia enhances the sugar uptake of the pancreatectomized dog's muscle three to fourfold (Levine et al.;[2] Issekutz et al.[3]).

On the other hand, little information is available regarding the effect of a sugar load on the hepatic glucose output in diabetes. Whereas in the normal organism, the liver seems to play an important role in reducing the arbitrarily elevated blood sugar level[4-6] it is not clear whether or not this function exists in pancreatectomized dogs lacking insulin. It was of interest, therefore, to investigate the effect of sugar infusion on the hepatic glucose output in the complete absence of functioning islet cells.

Two methods have been employed in these studies:

A. The disappearance rate of blood glucose was followed after a sugar infusion in normal and pancreatectomized dogs without functioning kidneys. The in situ measurement of the amount of glucose taken up by the skeletal muscles allowed an approximate estimation of the splanchnic sugar output or uptake.

B. The hepatic blood flow was measured directly and from the arteriovenous glucose differences the sugar output was calculated. The relationship between the splanchnic sugar output and the blood sugar level was investigated over the range of 60 to 700 mg./100 ml. in normal dogs and of 200 to 800 mg./100 ml. in pancreatectomized dogs.

From the Department of Physiology, University of Szeged, Hungary, and Department of Physiology, University of Greifswald, Germany. Present address of Dr. Issekutz: Lankenau Hospital, Division of Research, Philadelphia, Pennsylvania.

Part of this work was presented at the International Physiological Congress in Brussels in 1956 and a short summary of the whole work at the Annual Meeting of the Federation of the American Society for Experimental Biology, 1958.

METHODS

Group A:

The experiments were carried out on twelve pancreatectomized and six control dogs. Pancreatectomy was performed under aseptic conditions seven to fourteen days before the experiment. The maintenance dosage of Protamine Zinc Insulin was about 0.5 U/kg. once a day. That was withdrawn forty-eight to sixty hours before the experiment. On the day of the experiment, the dogs were given morphine (2 to 3 mg./kg.) subcutaneously, which was followed by an intravenous injection of chloralose (80 to 90 mg./kg.). The kidneys were removed or the ureters were ligated. Consideration of the uniform behavior of the nephrectomized and ureter-ligated animals, in all of the experimental series, indicated that it was not necessary to differentiate between the two groups.

The glucose uptake of muscles was determined by multiplying the blood flow by the arteriovenous sugar difference. Blood flow was measured in the vena profunda femoris by means of our formerly described method.[7] The blood in this vein comes primarily (90 to 95 per cent) from the musculature of the hind leg. After one or two hours' recovery period, the normal values were taken and, following this, the blood sugar level was adjusted by an adequate intravenous priming dose and a subsequent intravenous sugar infusion of two hours' duration, to levels of 366 to 1,105 mg./100 ml. in diabetic animals and to levels of 356 to 953 mg./100 ml. in normal animals. After stopping the infusion, the animal was observed for three to four hours.

If the glucose uptake of the examined muscle area is known, the amount of sugar taken up by the entire body musculature can be computed, assuming that all muscle takes up sugar at the same rate as the examined one. In this calculation, 50 per cent of body weight

was taken for the entire muscle weight of the body.[8] The gross amount of glucose disposed of by the organism can be calculated from the fall of the blood sugar level (mg./100 ml./min.) and the size of the extracellular compartment (25 per cent of body weight). Should this be greater than the amount taken up by the muscles, then the splanchnic area, first of all the liver, must also have taken up sugar. Conversely, if this value is lower than the amount of glucose taken up by the muscle, then glucose must have been released by the splanchnic area, most probably by the liver. The approximate sugar output (+) or uptake (—) of the splanchnic area can be estimated according to the simple equation:

$$\text{splanchnic sugar } \begin{matrix}\text{output}\\ \text{or}\\ \text{uptake}\end{matrix} \text{ (mg./min./kg. body weight)} = 5a - 2.5b$$

where "a" represents the glucose uptake of the muscle (mg./100 gm./min.) and "b" is the decrease of the blood sugar (mg./100 ml./min.).*

The first estimation of splanchnic sugar output or uptake, respectively, was done at the last minute of the sugar infusion when a more or less steady state of the blood sugar level was reached. In this case:

$$\text{splanchnic sugar } \begin{matrix}\text{output}\\ \text{or}\\ \text{uptake}\end{matrix} \text{ (mg./min./kg.)} = 5a - 2.5b - c$$

where "c" represents the amount of glucose mg./kg./min. infused.

It should be emphasized that this is an approximate gross calculation which does not include the sugar uptake of other tissues (brain, skin, bones). This would increase the value of 5a. The extracellular space could have taken 30 per cent of body weight which would have given a value of 3b instead of 2.5b. However, the observed differences between the pancreatectomized and control dogs, described below, were so striking that the error of this estimation seems not to affect the final conclusion.

Group B:

These investigations were carried out on thirty-two dogs of 12 to 25 kg. body weight. Twenty were pancreatectomized five to seven days previously. Insulin was withdrawn forty-eight hours before the experiment. Twelve dogs served as controls. The ureters were ligated in both pancreatectomized and control dogs to prevent the excretion of sugar and to achieve in the diabetic animals a fairly constant blood sugar level. The rate of the hepatic blood flow was measured as follows: A metal cannula with an attached rubber cuff was introduced into the jugular vein of dogs in morphine-chloralose anesthesia. It was passed through the vena cava superior and inferior until it reached the diaphragm (its position was checked by X ray). Simultaneously a heart catheter, equipped with a thin-walled rubber balloon on its end, was introduced into the left femoral vein. The catheter was passed through the inferior vena cava up to its junction with the hepatic veins. Inflation of the rubber cuff and the balloon with air forced the hepatic blood to flow through the metal cannula into a glass tube filled with physiological saline. This tube contained a glass ball which prevented the mixing of the blood with the saline, which streamed at the rate of the hepatic blood flow into a glass cylinder. The level of the glass cylinder could be adjusted to provide the necessary pressure gradient. The measurement was performed with the help of Anrep's hot-wire anemometer[9] as previously described.[7] After reading the deflection of the galvanometer and taking a sample from the hepatic blood, the normal circulation was re-established by reverting the blood which had been streaming through the cannula. The entire procedure required not more than forty seconds. Blood was taken simultaneously from the right femoral artery.

After determining the splanchnic sugar output or uptake, the blood glucose level was adjusted to 200 to 700 mg./100 ml. in normal dogs and to 300 to 800 mg./100 ml. in diabetic animals, by means of an adequate intravenous priming dose and a subsequent glucose infusion of ninety minutes' duration. The response of the liver was followed for ninety minutes in controls and for two hours in pancreatectomized dogs after stopping the sugar infusion.

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*The glucose uptake of 1 kg. muscle: 10a (mg./min./kg.) and that of the entire musculature (50 per cent of body weight: "W"):

$$10a \times 0.5W = 5aW$$

Glucose disappeared from 1 liter of the extracellular space was 10b (mg./min.) and from the entire extracellular space (25 per cent of body weight):

$$10b \times 0.25W = 2.5bW$$

An example:

In a control dog, at the end of the infusion, the sugar uptake of 100 gm. muscle was 2 mg./min. ("a"); the blood sugar decreased in the last thirty minutes of the infusion by 30 mg./100 ml. or 1 mg./100 ml./min. ("b"); the glucose infusion was 20 mg./min./kg. ("c"). Then the splanchnic sugar uptake $5 \times 2 - 2.5 - 20 = -12.5$ mg./min./kg. and sixty minutes after the infusion when the glucose dropped from 440 mg./100 ml. to 140 mg./100 ml. and the sugar uptake of the muscle was found 0.8 mg./100 gm./min. The estimated splanchnic sugar uptake was:

$$5 \times 0.8 - 2.5 \times 5 = -8.5 \text{ mg./min./kg.}$$

The blood glucose was determined according to Somogyi.[10] Statistical calculations were carried out according to Fisher.[11]

RESULTS

Group A:

Figure 1 shows the typical course of an experiment on a normal and on a pancreatectomized dog; both were nephrectomized. In the control dog, the blood sugar level fell quickly when the infusion stopped; in fact, it started to decrease before the end of infusion. Contrary to this, in the pancreatectomized animal the glucose level was steadily rising during the infusion and apart from minor oscillations it did not undergo any significant change during the first three or four hours of observation. The sugar uptake of the left hind leg muscles, at the end of the sugar load, was found to be elevated fivefold and about the same in both control and pancreatectomized dogs. However, at this time, the blood glucose concentrations were 420 mg./100 ml. in the control and 860 mg./100 ml. in the pancreatectomized animal. This seems to confirm the similar finding of Levine et al.[5] After the infusion, the sugar uptake of muscles of the control dog decreased as the blood glucose level fell, whereas in the pancreatectomized animal, the glucose uptake of the left hind leg showed a further increase. The calculated splanchnic response to the sugar load showed a marked difference between the normal and pancreatectomized dogs. Whereas in the normal animal the splanchnic area seemed to respond with a sugar uptake, in the diabetic organism the liver continued to release sugar even at a very high blood sugar level.

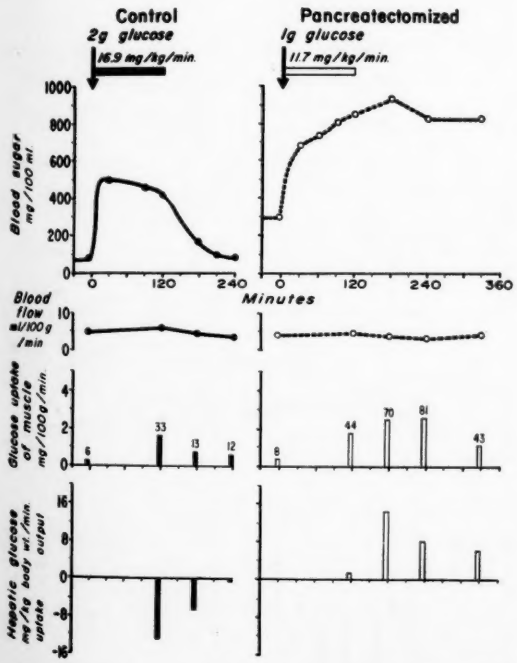

FIG. 1. Effect of sugar load on the peripheral sugar uptake in a control (weight 11 kg.) and in a pancreatectomized dog (weight 12 kg.) (both nephrectomized). Curves and columns from the top downward: blood glucose; blood flow in the muscle; glucose uptake of the muscle (figures above the columns show the arteriovenous sugar difference); approximative splanchnic sugar uptake or output, respectively.

Tables 1 and 2 summarize the results of eighteen similar experiments (twelve pancreatectomized and six controls). They are arranged according to the adjusted blood sugar levels (column 1). Column 3 represents the means (three or four determinations) of the estimated sugar output or uptake of the splanchnic area, *after the end* of infusion during the time intervals in-

TABLE 1

Estimated glucose output of the splanchnic area during hyperglycemia in depancreatized-nephrectomized dogs

Adjusted blood sugar level mg./100 ml.	Duration of observation. Minutes after the end of the glucose infusion	Estimated average glucose output mg./kg. body weight/min.
366	120	+3,5
390	210	—6,8 glucose uptake
443	150	+7,0
637	150	+6,0
638	210	+8,6
739	150	+9,1
865	240	+10,7
865	190	+9,9
870	180	+8,3
906	240	+4,9
1,050	150	+6,2
1,105	240	+12,2

TABLE 2

Estimated glucose uptake of the splanchnic area during hyperglycemia in nephrectomized normal dogs

Adjusted blood sugar level mg./100 ml.	Duration of observation. Minutes after the end of the glucose infusion	Estimated average glucose uptake mg./kg. body weight/min.
396	120	—3,8
417	120	—2,0
447	120	—3,8
507	150	—4,2
511	127	—6,6
953	180	—0,2

dicated by column 2. Nephrectomized diabetic control animals, without sugar load, showed a slight rise of 50 to 80 mg./100 ml. of the blood glucose level in four out of five cases. The fifth dog displayed between the third and fourth hours a sudden increase to above 500 mg./100 ml. blood glucose concentration. The blood sugar level of nephrectomized normal controls was reasonably constant (±5 mg./100 ml.) during the experimental period.

Hematocrit values were measured on sixteen animals, diabetic and control, in which the arbitrarily adjusted blood sugar level varied between 366 and 1,105 mg./100 ml. The mean value of the initial hematocrit was 0.39 ± 0.04. This fell to 0.33 ± 0.04 at the end of the infusion and remained unchanged to the end of the experiment. An average value of 0.33 ± 0.04 was obtained at 150 and 180 minutes, respectively.

Group B:

In continuation and extension of the experiments described under Group A, the splanchnic sugar output or uptake, respectively, was measured directly. Prior to the introduction of the sugar load, the blood glucose level in normal dogs ranged between 65 to 105 mg./100 ml. and in pancreatectomized between 230 and 350 mg./100 ml. In fifty-one observations on twenty normal control dogs (ureters ligated) the average sugar output of the splanchnic area was 6.5 ± 1.3 mg./100 gm. liver/min. or 1.7 ± 0.35 mg./kg. body weight/min. (table 3). The mean hepatic blood flow was 90 ± 7 ml./100 gm. liver/min. Lipscomb and Crandall[12] calculating the hepatic blood flow from the urea excretion found in normal unanesthetized dogs a hepatic glucose output of 2.0 ± 0.2 mg./kg. body weight/min. The glucose output of the splanchnic area seems to depend on the arterial blood sugar level (table 3). The correlation coefficient "r" = 0.640 is significant $p < 0.001$. It should be pointed out, however, that the extremely low sugar output measured at the higher arterial glucose levels (range 90 to 105 mg./100 ml.) was probably caused by an elevated sugar uptake of the intestinal tract. In pancreatectomized animals at an average blood sugar level of 283 mg./100 ml., the splanchnic glucose output was 5.1 ± 1.8 mg./100 gm. liver/min. or 1.9 ± 0.42 mg./kg. body weight/min. which was practically the same found in the controls at the average blood glucose concentration of 84 mg./100 ml. A similar observation was made by Crandall and Lipscomb[13] in pancreatectomized dogs and by Bearn, Billing, and Sherlock[14] in diabetic humans.

A sugar load in the form of an adequate infusion showed a marked difference between normal and pancreatectomized animals. Figure 2 shows two typical experiments. In the normal dog, the arbitrarily elevated blood sugar induced a marked sugar uptake of the splanchnic area. This is essentially the same phenomenon described at first by Soskin and co-workers.[4] No similar effect could be observed in pancreatectomized animals. In spite of the highly elevated blood sugar level (750 mg./100 ml.), the glucose output of the splanchnic area was continued and after the infusion, it seemed

TABLE 3

Splanchnic sugar output in normal dogs

Number of observations	Blood sugar range (mg./100 ml.)	Glucose output mg./min./100 gm. liver	mg./min./kg. body weight
19	65-80	14.5	3.34
11	80-90	5.6	1.73
21	90-105	0.8	0.25
Mean±S.E.	84.7±1.9	6.5±1.3	1.72±0.35

Correlation coefficient r = —0.640 p<0.001

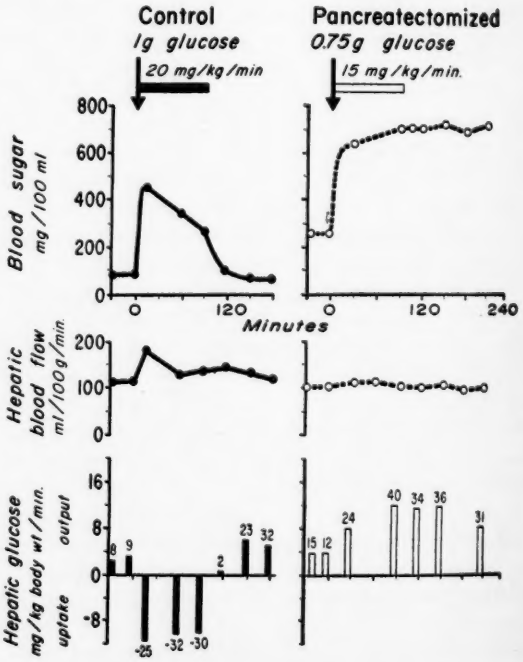

FIG. 2. Effect of sugar load in a control and in a pancreatectomized dog (both ureter ligated). From the top downward: blood sugar; hepatic blood flow (ml./100 gm. liver/min.); splanchnic sugar uptake or output, respectively. Figures above the columns show the hepatic arteriovenous differences.

to be rather increased. Here again as in Group A can be seen a pronounced difference in the rate of the elimination of sugar in the diabetic animal compared with the control dog.

Table 4 summarizes the results obtained at blood glucose concentrations, covering the range between 150 and 700 mg./100 ml. in normal and between 300 and 800 mg./100 ml. in diabetic dogs. The liver of the controls showed "the homeostatic function";[4] that is, a rather high sugar uptake at any level investigated. The deviation from the zero value is significant ($p < 0.01$) in all columns; the maximum is reached between 400 and 500 mg./100 ml. blood glucose. In contrast to this, the pancreatectomized animals did not show any well-pronounced splanchnic uptake of sugar comparable to the controls. In the range between 300 and 500 mg./100 ml., no consistent reaction was observed. In most instances sugar output was obtained but the deviation from the zero was not significant. Greater sugar loads which raised the blood level to 500 or 600 mg./100 ml. caused a marked and significant sugar output. The same is true for the ranges from 600 to 700 mg./100 ml. and from 700 to 800 mg./100 ml. This sugar output is as high or often even higher than that measured at 280 mg./100 ml. level.

DISCUSSION

Two methods have been used to demonstrate the effect of the elevated blood sugar upon the glucose output of the liver of pancreatectomized dogs. Group A, an indirect approach, gave qualitative rather than quantitative information. However, it had the advantage that apart from the ligation of ureters only a small operation (measuring the blood flow in the vena profunda femoris) was needed. The direct determination (Group B) of the hepatic blood flow yielded quantitative data but interfered even if for a short time, with the circulation of the vena cava inferior. It is important to note that both approaches gave essentially the same result, namely, the liver of pancreatectomized dogs put out sugar at arbitrarily elevated blood sugar levels which induced in every control a marked hepatic glucose uptake. Recently, Madison et al.[22] also reported that in pancreatectomized severely diabetic dogs with portacaval shunts, glucose infusion did not produce hepatic sugar uptake. In Group B, at blood sugar levels between 500 and 800 mg./100 ml. (table 3), the average hepatic glucose output of the pancreatectomized dogs was 27.3 mg./100 gm. liver/min. or 8.8 mg./kg. body weight/min. With the indirect method in Group A, at blood glucose concentrations between 443 and 1,105

TABLE 4

Effect of sugar load on the splanchnic glucose output (+) or uptake (—) blood sugar level mg./100 ml.

PANCREATECTOMIZED						
Range	150-300	300-400	400-500	500-600	600-700	700-800
Average blood sugar		351±8	456±7	546±7	640±9	734±12
Splanchnic output mg./min./100 gm. liver		5.2±3.6	9.9±5.5	29.0±8.4	19.6±4.9	37.7±10.9
mg./min./kg. body weight		1.8±1.0	2.8±1.8	8.5±2.1	7.6±2.0	12.1±4.6
t		1.43	1.81	3.45	3.99	3.43
n		15*	14†	15‡	21§	9‡
CONTROLS						
Average blood sugar	216	338	450	631		
Splanchnic uptake mg./min./100 gm. liver	—21.9±6.4	—22.5±6.1	—33.8±7.0	—29.9±4.7		
mg./min./kg. body weight	—5.7±1.8	—5.5±1.7	—9.1±2.1	—8.8±1.6		
t	3.40	3.67	4.79	6.33		
n	11‡	14‡	8‡	5‡		

Indexes mean the significancy of deviation from zero:
n = number of observations (two or three on the same dog)
t = significance of deviation from zero (Fisher)[21]

* $0.1 < p < 0.2$ † $0.05 < p < 0.1$ ‡ $p < 0.01$ § $p < 0.001$

mg./100 ml., the estimated hepatic output averaged 8.2 mg./kg./min.

The mechanism of this paradoxical hepatic response in diabetes needs further investigations. It is probable that the increased activity of adrenal cortex observed in pancreatectomized dogs[15] and in alloxan-diabetic rats[16] was involved in this phenomenon. Also, in humans, it was found that during diabetic coma the urinary excretion of ketosteroids may be considerably increased.[17] It was further shown that hyperglycemia induces an enhanced release of corticosteroids even in nondiabetic animals.[18–20] This may mean an enhanced gluconeogenesis and may explain the finding of Whittlessey and Zubbord[21] that glucose infusion tends to increase the plasma level of ketone bodies in pancreatectomized dogs. Also, Hetenyi and Forbath observed that the glucose load accelerates the increase of the nonprotein nitrogen in the plasma of pancreatectomized dogs.[22]

These observations suggest that a sudden increase of blood sugar (induced for instance by an abundant supply of carbohydrates) may have a "stresslike" effect in severe diabetes. Instead of depressing the hepatic glucose output, the sugar load increases glycolysis and gluconeogenesis, causing a "vicious circle" which may lead to a hyperglycemic coma especially in cases with low insulin sensitivity of the liver.[14]

SUMMARY

The splanchnic glucose output was investigated in pancreatectomized and nephrectomized or ureter-ligated dogs.

An indirect and direct method have been described. In the indirect method, the glucose uptake of the left hind leg muscles was measured in situ. The blood sugar level was adjusted to 366 to 1,105 mg./100 ml. by means of intravenous glucose infusion during a period of 120 minutes. After the infusion, the blood sugar was followed for three to four hours. From the changes of blood sugar level and the glucose uptake of musculature, the splanchnic glucose output was estimated. In a second series of experiments, the hepatic blood flow and the sugar output or uptake of the splanchnic area were directly measured through the right jugular vein. Changes of blood sugar level in the range between 65 and 105 mg./100 ml. showed a correlation with the splanchnic output of glucose in the control dogs. Elevation of the blood sugar levels by means of glucose infusion caused a sugar uptake in controls. No comparable sugar uptake was observed in diabetic dogs, where blood glucose levels above 500 mg./100 ml. increased markedly the splanchnic sugar output.

Both direct and indirect methods brought essentially the same results indicating that the "homeostatic function" of the liver was restricted in diabetes.

SUMMARIO IN INTERLINGUA

Le Effecto de Alte Nivellos de Sucro Sanguinee Super le Rendimento de Sucro Splanchnic in Diabete Experimental

Le rendimento splanchnic de sucro esseva investigate in canes panchrea- e nephrectomisate o uretero-ligate.

Es describite un methodo indirecte e un methodo directe. In le methodo indirecte, le fixation de glucosa per le musculos del gamba sinistro-posterior esseva mesurate in sito. Le nivello del sucro sanguinee esseva adjustate al nivello de 366 a 1.105 mg per 100 ml per medio de un infusion intravenose de glucosa durante un periodo de 120 minutas. Post le infusion, le sucro del sanguine esseva tenite sub observation durante tres a quatro horas. Super le base del alterationes del nivello de sucro sanguinee e le fixation de glucosa in le musculatura, le rendimento splanchnic de glucosa esseva estimate. In un secunde serie de experimentos, le fluxo de sanguine hepatic e le rendimento o le fixation de sucro per le area splanchnic esseva mesurate directemente via le vena dextero-jugular. Alterationes del nivello de sucro sanguinee in le region inter 65 e 105 mg per 100 ml revelava un correlation con le rendimento splanchnic de glucosa in le canes de controlo. Le elevation del nivellos de sucro sanguinee per le infusion de glucosa causava un fixation de sucro in le animales de controlo. Nulle comparabile fixation de sucro esseva observate in canes diabetic in que nivellos de sucro sanguinee de plus que 500 mg per 100 ml resultava in un augmento marcate del rendimento de sucro splanchnic.

Le duo methodos—le directe e le indirecte—produceva essentialmente le mesme resultatos. Isto indica que le "function homeostatic" del hepate es restringite in diabete.

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Diet and Glycoproteins

Although clinical studies of the influence of diet on the carbohydrate-containing proteins of serum (including effects of fasting and feeding of carbohydrate, protein or fat to normal individuals) have proved negative (Z. Stary, *Clin. Chem.* 3:557, 1957), rats have been found to respond to starvation (M. R. Shetlar and C. L. Shetlar, *Proc. Soc. Exp. Biol. & Med.* 88:622, 1955; H. E. Weimer and H. Nishihara, *Ibid.* 95:677, 1957) and protein depletion (H. E. Weimer and H. Nishihara, *loc. cit.*) with a decrease in the serum glycoprotein.

H. E. Weimer and H. Nishihara (*J. Nutrition* 67:137, 1959), have followed up this lead by exploiting the depletion-repletion technic of R. W. Wissler, R. L. Woolridge, C. H. Steffee, Jr., and P. R. Cannon (*J. Immunol.* 52:267, 1946). The animals were fed a protein-free diet until they had lost 25 per cent of their original weight. For repletion the diets contained 10 per cent vegetable oil, 4 per cent salt mixture (U.S.P. XIV) and a vitamin mixture, with the remaining 86 per cent made up of casein and starch. The percentages of protein varied as follows: 0, 8, 17, 27, 40 and 64 per cent.

Total serum glycoprotein, glycoprotein polysaccharide, seromucoid protein and seromucoid polysaccharide were determined. Albumin and globulin proteins were also measured with respect to amounts of protein and polysaccharide. Hematocrits and total hemoglobin were determined.

Hemoglobin and hematocrit values were in the normal range following depletion, but there was a pronounced hemodilution during repletion.

As far as weight repletion was concerned, the 40 per cent protein diet presented the best balance as shown by a more rapid repletion time and a higher food efficiency ratio even when compared to the 64 per cent protein diet.

Protein depletion caused significant reductions in all of the serum glycoproteins with the exception of the polysaccharide component of the albumin fraction. Following repletion on the 8 per cent protein diet, there was some increase in the globulin polysaccharide, but decreases were now observed in the seromucoid and albumin fractions. The 17 per cent protein diet restored total glycoprotein, albumin and globulin polysaccharide values to the normal levels, but there was no effect on the seromucoid fraction, which remained low.

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Special Article

Pancreatic Islet Histology and Carbohydrate Metabolism in Amphibians and Reptiles

Malcolm R. Miller, Ph.D., M.D., San Francisco

INTRODUCTION

The observations that amphibians may be deficient or lacking in pancreatic islet alpha cells, while reptiles and birds are very abundantly endowed with these elements, provides us with "natural experiments" that are both interesting and worthy of study. In particular, better understanding of the relationship of the pancreatic alpha cell to glucagon may result from the study of the carbohydrate metabolism of these vertebrate species. The present account will describe certain features of the histophysiology of the pancreatic islets and carbohydrate metabolism of amphibians and reptiles.

THE PANCREATIC ISLETS OF AMPHIBIANS

There are three major orders of living (modern) amphibians. These are the Apoda (Gymnophiona or Caecilia), the Urodela (Caudata or Salamanders and Newts), and the Anura (Salienta or Frogs and Toads). Of these, the pancreatic islets of a few species of urodeles (salamanders) and anurans (frogs and toads), but no apodans have been described.

The pancreases of amphibians are relatively poor in islet tissue as compared with other types of vertebrates and also have relatively small proportions of alpha cells, or even a complete absence of alpha cells.[1-3] While frogs and toads possess both alpha and beta cells, the proportion of alpha to beta cells is only 20 to 30 per cent alpha to 70 to 80 per cent beta cells.[4] Further, toads may possess a considerably larger portion of alpha cells than frogs.[5] Recent observations on the pancreatic islets of the frog, *Rana boyli*,[5] reveal very few alpha cells. Salamanders are extremely interesting in that no alpha cells can be demonstrated in the pancreatic islets.[1,6,7] It must be pointed out, however, that these generalizations are based on the study of very few species, and more extensive study may revise these opinions.

From the Department of Anatomy, University of California School of Medicine, San Francisco 22, California.

In our laboratory we have studied the pancreatic islets of several salamander species (*Taricha torosa, Amphiuma means, Ensatina eschscholtzi*) and all these possess pure beta cell islets.[5] Occasionally cells with clear nongranular cytoplasm are observed in the islets. These may be exhausted beta cells, or cells transitional between duct and islet cells, but demonstrate no alpha cell characteristics. The application of silver stains demonstrates no argentaffine elements in the amphibian pancreas.[8] Although other studies on urodelan islets are sparse, most observers describe only one type of granulated cell, the beta cell.[6,7]

In toads the pancreatic islets are composed of a thin peripheral rim of alpha cells surrounding the more abundant beta cells, while in frogs, the beta cells are arranged in elongate cords, with but few alpha cells separating the beta cell cords from the acinar tissue.[5]

If the alpha cell is the only, or the major source of glucagon, then the pancreatic islets of salamanders should be lacking in this hormone; the frogs have a very small amount, and the toads a small, but more significant quantity. Unfortunately, no biochemical studies have been reported on the pancreatic islets of various amphibian species and no direct evidence bearing on this relationship is available.

Indirect evidence, such as the relative sensitivity to glucagon of various vertebrate species is as yet insufficiently studied and the results inconclusive. In salamanders, relatively large dosages (200 μg. per kilogram) of amorphous glucagon administered intraperitoneally failed to evoke a rise in blood sugar but intracardiac injections of crystalline glucagon (10 μg. per kilogram) stimulated a mild rise in blood sugar.[5] By contrast, the bullfrog is reportedly very sensitive to glucagon.[8] Sensitivity or insensitivity to glucagon, however, may bear no relationship to natural glucagon content. Whether amphibia are less sensitive to glucagon, or contain less in their pancreases than other vertebrates remains to be determined.

The beta cells of the pancreatic islets of amphibians respond to hyperglycemia provoking agents as do the beta cells of other vertebrate animals. Hyperglycemia produced by glucose, adrenalin, or hydrocortisone administration results in beta cell hypertrophy and degranulation.[1] Procedures lowering the blood sugar such as insulin administration or hypophysectomy result in a decrease in beta cell volume and clumping or loss of beta cell granules.[1]

The results of alloxan administration are variable. In most cases, alloxan has been ineffective in destroying the beta cells of the pancreatic islets of amphibians.[1,2,9] Recent observations in this laboratory, however, show that recrystallized alloxan given intravascularly is more effective and less toxic than impure alloxan given by other routes.[5]

Synthalin A or cobaltous chloride had no effect on the pancreatic islets of salamanders.[1]

THE PANCREATIC ISLETS OF REPTILES

Most modern reptiles are represented by the Chelonia (turtles), Squamata (lizards and snakes), and the Crocodilia (crocodiles and alligators). Studies on various species of reptiles have indicated that there is considerable difference in the pancreatic islet morphology of reptiles of different orders. The squamate reptiles (lizards and snakes) characteristically have large islets primarily localized in the splenic portion of the pancreas.[2,10-12] These large islets are composed of both alpha and beta cells so mixed that no particular pattern of cell distribution is apparent. The proportion of alpha and beta cells is about equal or sometimes there are more alpha cells.[10,12] The alpha cells of lizards and snakes are densely packed with granules that stain brilliantly with azocarmine or phloxine. The large size of the islets together with the ease with which the alpha and beta cells may be differentiated renders the lizard and snake pancreas a very desirable object for histophysiologic investigation.

The pancreatic islets of turtles are not as large as those of lizards and snakes, nor are they as confined to the splenic tail.[6] The alpha cells also are peripheral in distribution, whereas they are not in lizards and snakes. I know of no descriptions of the pancreatic islets of alligators or crocodiles.

An observation of unknown significance is that the beta cell granules of amphibians are more easily stained with aldehyde fuchsin, while those of reptiles are more easily stained with chrome alum hematoxylin. Only a few reptilian alpha cells are stained by silver methods and thus a positive argentaffine reaction is not a necessary characteristic of the reptilian alpha cell.[10]

The relatively large numbers of alpha cells in the islets of lizards and snakes were thought to be associated with a large content of pancreatic glucagon.[10,11] This idea was corroborated by a similar situation in some birds where a large alpha cell population coexists with a high glucagon content (six to ten times that of mammals).[14] In reptiles, unfortunately, no biochemical studies have been reported and the pancreatic glucagon content is unknown. Only on the basis of some indirect evidence may this hypothesis be entertained (*vide infra*).

The beta cells of the reptilian pancreas hypertrophy and degranulate subsequent to hyperglycemia produced by glucose, adrenalin, or hydrocortisone administration.[10,11] The alpha cells appear to be overstimulated by chronic insulin injections.[10,11]

Alloxan, if given in large dosages, and over an extended period of time produces beta cell damage and hyperglycemia.[9,10] Synthalin A evokes some alpha cell degranulation and destruction, but of insufficient extent to evaluate properly the role of the alpha cell in the regulation of the blood sugar level.[10]

NORMAL BLOOD SUGAR VALUES IN AMPHIBIANS AND REPTILES

In both anuran and urodelan amphibians, normal blood sugar values range from zero[8] to 75 mg. per 100 ml.[15] Average values are more of the order of 30 to 35 mg. per 100 ml.[8] As compared with normal blood sugar values of reptiles, birds, and mammals, these are relatively low levels. Whether these lower values are associated with a low glucagon content of the pancreas is as yet unknown. That no correlation exists between the normal blood sugar level and the amount of alpha cell tissue is indicated by the observation that non-alpha-cell-containing urodeles have no lower blood sugar levels than do alpha-cell-containing toads. Furthermore, fish that are adequately endowed with alpha cells demonstrate relatively low normal blood sugar levels.[8]

The lower normal blood sugar of amphibians may be related to other factors such as a lesser demand for glucose by these animals as compared with reptiles, birds, and mammals, and not so much with the insulin-glucagon balance. The fact that higher blood sugar values are noted during the breeding season[1,18] correlates not only with the time that the animals are physically very active, but also with the time of increased endocrine activity.[17,19] That temperature may not be a factor normally regulating the blood sugar level

is attested by the observation that breeding in many amphibians occurs during a cooler spring, while during the hotter summer the blood sugar is lower.

While considerable range of normal blood sugar values has been reported for various species of reptiles (30 to 205 mg. per 100 ml.), average values are usually in the range of 90 to 100 mg. per 100 ml.[1,2,21–28] As in the amphibia, there is no correlation between the blood sugar level and the relative amounts of alpha and beta cells in the pancreatic islets. The higher blood sugar levels of reptiles as compared with the amphibia may possibly indicate a greater need for glucose by reptiles. In this regard it may be significant that birds which have high energy requirements,[24] also have high normal blood sugars (150 to 250 mg. per 100 ml.).[25] As in many amphibians, north-temperate-living reptiles have higher blood sugar levels during the breeding season. Tropical living reptiles, on the other hand, may not show seasonal blood sugar variations.[26]

Coincident with the higher peripheral blood sugar at the time of breeding, the liver glycogen and the fat body reserves are low.[20] In the fall, when most of the north temperate species are mobilizing fat and carbohydrate for gonadal reconstitution and fat body storage, the peripheral blood sugar is low.

EFFECT OF NUTRITIONAL STATUS AND GLUCOSE ADMINISTRATION

In amphibians, meals of natural food do not cause much variation in the blood sugar.[8] Reptiles, on the other hand, tend to exhibit hyperglycemia of several days' duration following a meal.[10] Whether this difference is related to the type of food, the rate of digestion and absorption, to variations in the temperature at which these processes occur, or to basic metabolic differences, is not yet known.

Certainly temperature plays a role in decreasing the time required for many metabolic processes to transpire,[27] but specifically which processes are affected is little studied or known.

In nature, both amphibians and reptiles are capable of undergoing long periods of fasting. Approximately two months' starvation in the laboratory (at temperatures of approximately 70°F.) is required before the blood sugar levels become significantly hypoglycemic.[20,28]

Orally administered glucose is absorbed and assimilated much more slowly in amphibians and reptiles than in mammals. Increasing the temperature will hasten the process, but still several days are required for re-establishment of a normal blood sugar after glucose administration.[27]

THE EFFECTS OF INSULIN AND GLUCAGON ADMINISTRATION

Since only the effects of mammalian insulin and not more closely related types of insulin are known in the amphibians and reptiles, conclusions concerning the reactions of these species to insulin must be tentative. Further, the concepts of "insulin sensitivity" and "insulin resistance" may not be too significant in light of our ignorance of the specific role(s) of insulin in carbohydrate metabolism in either mammals or the lower vertebrates. Regardless of the specific ways in which amphibians or reptiles respond to mammalian insulin, one outstanding difference in the behavior of these species as compared with mammals is the very long period of time required for amphibians and reptiles either to respond initially to insulin or for metabolic homeostasis to be re-established.

In amphibians, mammalian insulin produces marked blood sugar changes when approximately 10 units per kilogram are given.[1,29,30] Unfortunately minimum effective dosages have not been determined. With dosages ranging from 10 to 50 units per kilogram, there is no response for an hour or more. As contrasted with reptiles, there is no initial hyperglycemic phase, but the gradual development of a severe hypoglycemia lasting several days.[1,29] With dosages less than 50 units per kilogram, most animals recover a normal blood sugar level in three to seven days. Larger dosages (greater than 50 units per kilogram), however, most frequently result in death. Shock symptoms, such as muscular weakness, incoordination, trembling, or spasticity, will appear immediately if dosages of approximately 1,000 units per kilogram are given, but are delayed in appearance for several days if dosages of 50 units per kilogram are administered.[1] Insulin shock in amphibians, as well as reptiles, need not be related to hypoglycemia, as severe shock may occur when the blood glucose is not depressed.[1,20,29] Many of the effects of insulin in the cold blooded vertebrates are certainly due to direct stimulation of the central nervous system.[29]

If 1 to 2 units per kilogram of mammalian insulin are administered to turtles,[22] or even some snakes, hypoglycemia of several hours' duration occurs. The minimum blood sugar affecting dose in alligators is approximately 10 units per kilogram.[31] While these small dosages of insulin do not evoke a hyperglycemic phase preceding the hypoglycemic response, larger dosages (10 units per kilogram in the turtle;[5] 100 units per kilogram in the alligator;[31] and probably more than 100 units per kilogram in lizards[5]) produce a hyper-

glycemic phase of several hours' duration preceding an ultimate hypoglycemia of several days' duration. The initial hyperglycemic phase following large dosages of insulin has been attributed to the hyperglycemic factor contaminant in mammalian insulin preparations.[10,17] Recent observations in my laboratory, however, have demonstrated that glucagon-free insulin is equally provocative of hyperglycemia in turtles. Thus, the hyperglycemic response of reptiles to large dosages of mammalian insulin must be explained on a basis other than glucagon contamination.

That the alpha-cell-abundant and possibly glucagon-rich pancreatic islets of reptiles play a "protective" role against insulin is also questionable in light of the observation that pancreatectomized lizards also become hyperglycemic after insulin administration.[10] Factors other than pancreatic, such as adrenal medullary response, direct or indirect nervous system effects, or other endocrine effects must be responsible for the hyperglycemic effect of insulin and the apparent insulin resistance of reptiles.

EFFECTS OF EXTRAPANCREATIC HORMONES

In all species of amphibians and reptiles so far studied, epinephrin and hydrocortisone evoke significant elevations in blood sugar.[1,2] Anterior pituitary hormones, either crude or purified, are effective in elevating blood sugar in pancreatectomized animals, but not in normal animals.[9] The relative roles played by the products of the pituitary, thyroid, and interrenal gland hormones in the regulation of blood sugar in the lower vertebrates have not been adequately studied.

THE EFFECTS OF ABLATION OF THE PANCREAS, ANTERIOR PITUITARY, AND INTERRENAL GLANDS

Total pancreatectomy produces a diabetic syndrome in all species of amphibians. In frogs and toads (anurans), the hyperglycemia, glycosuria, and polyuria following pancreatectomy can be readily alleviated by hypophysectomy or interrenalectomy (adrenal cortical removal).[9] In salamanders (urodeles) by contrast, hypophysectomy subsequent to pancreatectomy will not alleviate hyperglycemia. Only after interrenal atrophy subsequent to long-standing hypophysectomy (several weeks), will the hyperglycemia of pancreatectomy be reduced.[1,2] At the present time, one may postulate that the interrenal gland of urodele amphibians (salamanders) are much less pituitary dependent than the interrenal glands of anurans (frogs and toads), or than any other so far studied vertebrate species.

Hypophysectomy alone results in a mild decline in blood sugar.[1,8] The interrenal glands, because of their location within the mesonephric capsule in amphibians, are extremely difficult to remove without severe injury to the kidneys and vena cava. However, both direct and indirect evidence indicates that the interrenal glands play a prominent role in maintaining the blood sugar of amphibians.[1,8]

In turtles, pancreatectomy is followed by almost immediate hyperglycemia and the ultimate development of other diabetic signs,[8,2] while in snakes and lizards, hypoglycemia of several days' duration precedes the development of hyperglycemia.[9,10] It is possible that the hypoglycemia following pancreatectomy in the lizard results from the removal of a pancreas rich in glucagon in an animal dependent on this hormone for maintenance of its blood sugar. This possibility was corroborated by Mialhe's observations[14] of a like state of affairs in the duck. The more usual behavior of the turtle in becoming immediately hyperglycemic after pancreatectomy might be related to the observation that turtle pancreatic islets appear morphologically much less rich in alpha cells than those of lizards and snakes. Unfortunately, we know nothing of the alligator in this regard. While the observations that certain types of reptiles and birds become hypoglycemic soon after pancreatectomy are very interesting and provocative, the explanation of this behavior, though it may be related to glucagon-insulin balance, awaits further study and explanation.

Hypophysectomy effects but a mild decrease in blood sugar in reptiles, while hypophysectomy after pancreatectomy is sometimes effective in lowering the hyperglycemia.[8,2] The intimate association of the interrenal gland with the wall of the inferior vena cava makes removal of this adrenal cortical homologue extremely difficult, and thus the role of this gland in the regulation of carbohydrate metabolism is judged important on the basis of indirect evidence alone.

CONCLUDING REMARKS

While in many ways the regulation of carbohydrate metabolism is similar in amphibians and reptiles and mammals, there are certain apparent differences, which when further studied and explained may greatly aid in extending our knowledge of carbohydrate metabolism in man and mammals. These differences and their possible significance are as follows:

1. The time required for response to blood sugar altering stimuli and for the re-establishment of metabolic homeostasis after changes have been produced is much greater in amphibians and reptiles than in birds

and mammals. This is probably associated in part with the higher temperatures at which these processes normally occur in warm blooded animals, but also may be due to different metabolic mechanisms.

2. Some species of urodelan, but not anuran amphibians, appear to lack alpha cells in the pancreatic islets and to be relatively insensitive to glucagon. The absence of alpha cells in the urodelan pancreas may be correlated with an absence of glucagon. That glucagon is, or is not present, in the pancreases of either urodeles or anurans remains to be determined.

3. Squamate reptiles (snakes and lizards) appear to possess relatively large amounts of alpha cells in the pancreatic islets. Whether this is correlated with a large glucagon content remains to be determined. That such a correlation has been established in the duck, renders the possibility likely in lizards and snakes where both insulin resistance and immediate hypoglycemia following pancreatectomy have also been observed.

4. Reptiles seemingly tolerate very large dosages of mammalian insulin. This is particularly true of lizards, but less so for snakes, alligators, and turtles. That the "insulin resistance" is due to a large content of counteracting glucagon is unlikely in view of the observation that pancreatectomized lizards are also insulin resistant. That the glucagon contaminant of mammalian insulin preparations is the protecting factor is unlikely, as glucagon-free preparations are also hyperglycemic in effect as well as tolerated in large dosages. It must be considered that insulin probably stimulates the central nervous system which may have both direct and indirect effects on regulation of carbohydrate metabolism.

5. While hypophysectomy will alleviate hyperglycemia following pancreatectomy in all so far described vertebrates, it will not in urodele amphibians. The interrenal tissue of urodele amphibians seems to be much less pituitary dependent than that of other animals, and will thus maintain a diabetic hyperglycemia in the absence of the pituitary gland. After long standing hypophysectomy, the interrenal glands of urodeles will atrophy, and then the hyperglycemia of pancreatectomy will be alleviated.

SUMMARIO IN INTERLINGUA

Le Histologia del Insulas Pancreatic e le Metabolismo de Hydrato de Carbon in Amphibios e Reptiles

Durante que in multe aspectos le regulation del metabolismo de hydrato de carbon es simile in, de un latere, le amphibios e reptiles e, del altere latere, le mammales, il etiam existe certe apparente differentias, le quales —quando illos es studiate additionalmente e explicate definitemente—promitte devenir de grande adjuta in le extension de nostre cognoscentias del metabolismo de hydrato de carbon in humanos e altere mammales. Iste differentias e lor signification possibile es le sequentes:

1. Le tempore requirite pro le declaration de un responsa a stimulos alterante le sucro del sanguine e pro le restablimento de homeostase metabolic post que alterationes in le sucro del sanguine ha essite effectuate es multo plus longe in amphibios e reptiles que in aves e mammales. Iste facto es probabilemente associate in parte con le temperatura plus elevate al qual iste processos occurre normalmente in animales de sanguine calide, sed il es etiam possibile que illo es le resultato de differente mechanismos metabolic.

2. Certe species de urodelos, sed non de anuros, pare haber nulle cellulas alpha in le insulas pancreatic e esser relativemente insensibile al effecto de glucagon. Le absentia de cellulas alpha in le pancreas urodelic pote esser correlationate con le absentia de glucagon. Si o non glucagon es presente in le pancreas de urodelos o in le pancreas de anuros remane a determinar.

3. Le reptiles squamate (serpentes e lacertas) pare posseder relativemente grande quantitates de cellulas alpha in le insulas pancreatic. Si o non iste phenomeno es correlationate con un alte contento de glucagon remane a determinar. Le facto que un tal correlation esseva establite in le anate rende probabile que le mesmo vale pro lacertas e serpentes in que on ha observate resistentia a insulina e le disveloppamento immediate de hypoglycemia post pancreatectomia.

4. Reptiles pare tolerar grandissime doses de insulina mammalian. Isto es particularmente ver pro lacertas. Le observation es minus frappante in le caso de serpentes, alligatores, e tortucas. Que le "resistentia a insulina" esserea un effecto de un grande contento de glucagon contra-active es pauco probabile in vista del observation que pancreatectomisate lacertas es etiam resistente a insulina. Que le glucagon contaminante le preparatos de insulina mammalian esserea le factor protectori es improbabile proque preparatos sin glucagon etiam exerce un effecto hyperglycemic e es tolerate in grande doses. On debe considerar le possibilitate que insulina stimula le systema nervose central e que isto exerce effectos tanto directe como etiam indirecte super le regulation del metabolismo de hydrato de carbon.

5. Durante que hypophysectomia allevia le hyperglycemia occurrente post pancreatectomia in omne le vertebratos usque nunc describite, iste phenomeno non

se observa in amphibios urodelic. Le tissu interrenal de amphibios urodelic pare depender multo minus ab le pituitario que le correspondente tissu in altere animales e pote, per consequente, mantener un hyperglycemia diabetic in le absentia del glandula pituitari. Post prolongation del stato hypophysectomic, le glandulas interrenal de urodelos deveni atrophic, e alora le hyperglycemia causate per pancreatectomia es alleviate.

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Special Article

Bio-assay of Insulin and Insulin-like Activity

Report on a Seminar

DR. A. BAIRD HASTINGS (Conference Chairman) (*La Jolla, California*) opened the meeting. He pointed out that insulin in serum is essentially a trace protein, occurring in a molecular ratio of 1:2,000,000 among other protein molecules, which makes its analysis and quantitation a difficult task.

DR. ALBERT RENOLD (*Boston*) began by briefly describing the method and materials used in the rat epididymal fat pad assay for insulin-like activity (ILA), using $C^{14}O_2$ production from glucose-1-C^{14} as an index of ILA. Results are expressed as the logarithm of the counts per minute per milligram of wet adipose tissue, a reference measurement which correlates well with the nitrogen content of the fat. Normal fasting values with this method average 250 microunits per milliliter and range from thirty to 500. DR. MINDEL SHEPS (*Boston*) discussed the statistical analysis of the values obtained using this assay method. She explained why the log dose, log response curve gave the most reliable results, and then outlined the statistical treatment necessary to calculate the limits of confidence of any given assay. She then showed that analysis of 103 assays in Dr. Renold's laboratory had revealed a mean lambda of 0.32. Also, repeated assays of the same sera have given results not significantly dissimilar. She emphasized that differences within the same assay, particularly as regards sera from the same donor, are much easier to detect than when different assays are compared.

DR. FRED GOETZ (*Minneapolis*) then discussed his technic for measuring hypoglycemia in vivo, using intact female mice of the DBA-1 strain. With cyclic lighting, the blood sugars tend to fall in the same range at any given time and therefore only the blood sugar following administration of an insulin standard or a serum sample need be measured. This assay is sensitive in the range of 1.2-2.0 mu/ml. Although the lambda averages 0.78 the confidence limits are fairly close since large numbers of animals can be used. The method has the advantages of (1) measuring the best-known effect of insulin, (2) using units that have pharmacological significance, and (3) responding appropriately to dilution of the unknown substance. It is, however, cumbersome and not sensitive enough to detect serum insulin in the physiologic range. DR. EUGENE JOHNSON (*Minneapolis*) elaborated further on the response curve obtained with this method and pointed out reasons why the most dependable segment of the curve fell between 1.2 and 4.0 mu/ml.

DR. PAUL BEIGELMAN (*Los Angeles*) briefly summarized his experience with two in vivo assay methods and with the adipose tissue method modified to measure glucose disappearance. He listed several points which seemed crucial for best results: animal weight, weight of adipose tissue segments, concentration of glucose in medium, etc. Addition of varying amounts of protein to the buffer made little difference in his results. Normal values with this method agreed in general with those reported by Drs. Renold and Sheps.

DR. HOLBROOKE SELTZER (*Dallas*) compared values for fasting and postglucose insulin-like activity obtained by various workers using the rat diaphragm method. Some of the variations may be related to modifications of diaphragm technic. Dr. Seltzer felt that the workers who used diluted plasma or serum obtained results which compared well with those from undiluted specimens provided they did not correct for the dilution. When glucose uptakes in the various laboratories were compared, and levels of insulin-like activity were all calculated from a single curve, five out of the six workers came up with normal values in the same range, viz., from 50 to 100 microunits per ml. It is not known whether the authors referred to would agree with this recalculation.

DR. EVELYN ANDERSON (*Bethesda*) described her in vivo assay based on the production of hypoglycemia in fasting hypophysectomized male rats. It is sensitive to the range of 1-2 mu/ml. with a lambda of 0.36. Using protein extracts prepared by Dr. Bates, she is able to detect values within the normal range. Recovery of I^{131}-labeled insulin added to the serum prior to frac-

This study is a report on a seminar held under the auspices of the Metabolism Study Section, National Institutes of Health, in Los Angeles, California, on Jan. 21, 1960.

tionation revealed good recovery of both the radioactivity and the insulin activity. Normal values with this method in dogs averaged 50 microunits per ml. Higher values were observed in pancreatic vein blood (up to 500 mu per ml.).

DR. ERIC BALL (*Boston*) reported on the possible use of net CO_2 formation by epididymal adipose tissue for the assay of ILA, since the respiratory quotient of adipose tissue actively synthesizing fat from glucose greatly exceeds unity. A specific advantage of this procedure derives from its simplicity and the ability to compare the activity of the insulin stimulated tissue with the *same* tissue prior to the addition of insulin or of the solution to be tested and after maximal stimulation by added insulin. ILA of any unknown solution can be expressed in per cent of maximum possible stimulation for each individual tissue segment.

DR. JOHN LEONARD (*Cleveland*) pointed out that the metabolic effects of serum ILA and insulin upon rat adipose tissue were remarkably similar with one exception: ILA and insulin varied in their effects upon glycogen synthesis by this tissue. Furthermore, ILA measured by the adipose tissue technic could not be extracted by methods which do recover small quantities of added crystalline insulin. Whereas purified albumin and gamma globulin do not affect adipose tissue, a number of factors such as cationic composition, methylene blue and Ethylenediamine Tetraacetate (EDTA) do. He found ILA to be essentially unchanged in totally depancreatized dogs, up to one week after operation, a result which throws doubt on the specificity of this method on the state of insulin in plasma or both.

DR. JOHN OWEN (*Washington, D. C.*) underlined the need to keep glucose-C^{14} solutions frozen, showing that bacterial contamination produced spontaneous liberation of $C^{14}O_2$ even in the absence of fat. When this occurred, the slope of the standard curve was usually flat. He also showed that preincubation overnight of buffer and insulin with reduced glutathione, 0.02M, resulted in complete disappearance of insulin-like activity. Sera similarly treated showed marked inhibition of activity compared to untreated aliquots of the same sera. He suggested that such treatment may destroy any effects due to insulin itself, and that the remaining activity might be due to nonspecific protein or ionic effects.

DR. CHARLES READ (*Iowa City*) commented on the apparent rise of ILA in sera diluted increasingly with buffer. This was known to be true for ILA as measured with the hemidiaphragm technic, but was also observed with adipose tissue, although contradicting experience was also reported. DR. NORMAN ALTSZULER (*New York*) has begun studies in order to test the possible usefulness of streptokinase in the measurement of ILA, since it induces the formation in serum of plasmin, which has been reported to degrade growth hormone, ACTH and glucagon but *not* insulin. Early experiments appeared encouraging. DR. HENRY LEE (*Indianapolis*) demonstrated that nicotinic acid (10^{-3}M) exerted a characteristic insulin-like activity upon adipose tissue, including accelerated lipogenesis. This seemed a rather clear demonstration of the need to term activities measured with assays of this type "insulin-like," rather than due to insulin.

DR. ROSALYN YALOW (*New York*) described the *immuno-assay* technic used by herself and Dr. Berson. This elegant technic makes use of paper electrophoresis in order to separate free from antibody-bound insulin-I^{131} when incubated with antiserum to beef insulin obtained in guinea pigs. She emphasized the importance of using standard curves obtained with insulin of the species studied, whenever insulin measurements are sought in that species. Normal fasting values in man averaged 25-30 microunits per ml., higher levels being obtained after glucose administration and in patients with islet-cell tumors. Increases were also observed with tolbutamide and after the administration of leucine to leucine sensitive infants. A delayed but prolonged insulin response to glucose was observed in untreated diabetic individuals of the maturity-onset type. DR. GERALD GRODSKY (*San Francisco*) reported on an immuno-assay similar to that of Yalow and Berson which uses, however, differential salt precipitation to distinguish "free" from "bound" insulin I^{131}. Furthermore, he subjects plasma or serum samples to acid-ethanol precipitation and extraction prior to assay. With this technic and using I^{131}-labeled insulin of low specific activity, he was unable to detect measurable amounts of insulin in normal serum (i.e., less than 20 microunits per ml.), found from 50 to 200 microunits after glucose and had quantitative recoveries from human pancreatic tissue. DR. EDWARD ARQUILLA (*Los Angeles*) recalled his results with a different immuno-assay technic using agglutination and hemolysis of sensitized red blood cells as index of the insulin reaction with insulin anti-serum obtained in rabbits. With his technic insulin levels in serum appeared to be exceedingly high, of the order of 3,000 microunits per ml. He generally emphasized the importance of keeping in mind that immunological technics are based on the

assumption that circulating insulin is immunologically identical with insulin extracted from pancreas by quite drastic procedures.

DR. PAUL BEIGELMAN (*Los Angeles*) reviewed the evidence indicating that ILA in serum is present in Cohn Fractions II and III and PGP, using differential precipitation technics; primarily in the region of beta and alpha globulins, using electrophoretic separation. The importance of the conditions used for either precipitation or electrophoresis was emphasized. DR. HARRY ANTONIADES (*Boston*) discussed extensive studies indicating that a major part of insulin in serum, and also in pancreas, is present in the form of a complex with other proteins, probably of a basic nature. His results indicated that this combination might be rather specific, possibly involving a single, specific basic protein. He also presented evidence suggesting that insulin present in this form does not react in characteristic fashion with antiserum prepared against crystalline insulin. Much of his evidence was based upon elaboration of the observation that insulin from serum and pancreas is in large measure adsorbed on cationic exchange resin under conditions precluding the adsorption of free, crystalline insulin. DR. KARE GUNDERSON (*Boston*) reported that extracts from serum or pancreas which exhibited no ILA by the diaphragm method could be activated by extraction at *p*H 10. The extracts were active toward adipose tissue even before precipitation. These findings again suggest the existence of different forms of serum insulin, characterized by differences in their isoelectric point and in their biological activity toward different tissues.

DR. HIROMICHI NARAHARA (*St. Louis*) discussed his studies on the interaction of insulin from several species with antiserum to beef insulin obtained in rabbits. In the presence of antiserum, insulin-I^{131} degradation in vitro was markedly slowed, indicating that the antiserum interfered with the proteolytic degradation of insulin. Antiserum to beef insulin interfered with the effect upon diaphragm of insulin obtained from the pancreas of several species, including man and rabbit. Furthermore, antiserum to beef insulin neutralized endogenous ILA (diaphragm) of serum obtained from beef, pig, rabbit (not in all instances), and man.

DR. ROBERT BATES (*Bethesda*) discussed his results with a dry to wet extraction procedure of lyophilized plasma, a procedure he first used for the concentration of TSH. In collaboration with Dr. Anderson, he regularly achieved approximately fifty-fold concentration of plasma ILA, as measured in vivo. DR. JAMES FIELD (*Bethesda*) reported that he obtained normal values in the range 0 to 65 microunits per milliliter using the hemidiaphragm as test tissue and glycogen deposition as index of ILA. He also reported evidence from one patient suggesting that antiserum to beef insulin obtained in man (from an insulin resistant patient) neutralized endogenous human ILA.

DR. RICHARD EGDAHL (*Richmond, Virginia*) reported on ILA measurements carried out in dogs with an adipose tissue technic similar to that described by Dr. Renold. ILA was uniformly higher (ten to fifteen times) in pancreatic vein blood than in blood obtained from any other site. However, he confirmed Dr. Leonard's observation concerning persistence of ILA in totally pancreatectomized dogs. It appeared from his experiments that the liver, under certain conditions, may be able to store insulin.

The recorders, Drs. Albert Renold and John A. Owen, Jr., suggest the following summary with interpretation.

SUMMARY

1. Any bio-assay stands four-square on the basic requirements of specificity, sensitivity, reproducibility, and practicality. Since insulin has multiple biologic activities, a multiplicity of bio-assays is possible and, indeed, desirable. In our present state of knowledge, each specific method fulfills some requirements, though often at the expense of others; the variety of methods which have been developed and are being developed should permit careful cross-checking on these points, and thus contribute to methodical progress in the study of insulin secretion, distribution and action.

2. Immuno-assay technics represent a new, important and exciting development in the field of insulin assay. The generally lower fasting values obtained by this technic probably represent a high degree of specificity for insulin per se.

3. Accumulating evidence suggests that insulin may be present in serum and in pancreas in more than one chemical form or combination. At least one of these forms of endogenous insulin appears to differ rather markedly from crystalline insulin extracted from beef pancreas. It is likely that in the future we shall have to consider complex interactions between insulin and insulin "binding" or "carrying" proteins, a development in line with what is being uncovered with regard to other hormones as well.

4. Although any insulin-like activity of serum which might be proved noninsulin in nature is likely to be considered an interference and a nuisance, it should be considered also as an interesting object of study.

SUMMARIO IN INTERLINGUA

Reporto Relative a Un Seminario Concernite con Insulina e Activitates Simile a Illo de Insulina

1. Omne bio-essayo debe satisfacer le requirimentos fundamental de specificitate, sensibilitate, reproducibilitate, e practicalitate. Viste que insulina ha multiple activitates biologic, un multiplicitate de bio-essayos es possibile e, de facto, desirabile. In le stato presente de nostre cognoscentias, omne methodo individual satisface certe requirimentos, ben que frequentemente al expensa de alteres. Le varietate del methodos que ha essite disveloppate e que continua esser disveloppate permitte le meticulose scrutinio comparative de tal questiones e promitte assi contribuer al progresso methodologic del studio del secretion, distribution, e action de insulina.

2. Technicas de immuno-essayage representa un nove, importante, e promittente disveloppamento in le campo del essayage de insulina. Le generalmente plus basse valores jejun que es obtenite per iste technicas representa probabilemente un alte grado de specificitate pro insulina per se.

3. Datos de plus in plus massive suggere que insulina es presente in le sero e in le pancreas in plus que un forma e combination chimic. Al minus un de iste formas de insulina endogene pare differer satis marcatemente ab insulina crystallin que es extrahite ab le pancreas bovin. Il es probabile que in le futuro nos va deber considerar complexe interactiones inter insulina e proteinas "ligante" o "portante" insulina, un disveloppamento de accordo con le constatationes currente in studios de etiam altere hormones.

4. Ben que omne activitate seral que es simile al activitate de insulina va probabilemente esser considerate como un phenomeno disturbatori e enoiose si tosto que on ha demonstrate que il non se tracta de insulina, tal agentes deberea esser considerate como digne de interesse e de investigationes special.

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ALBERT E. RENOLD, M.D.
JOHN A. OWEN, JR., M.D.
PETER H. FORSHAM, M.D.
Recent Chairman, Metabolism Study Section,
National Institutes of Health

EDITORIAL

EXACERBATION OF DIABETES BY EXCESS INSULIN ACTION

Insulin therapy of diabetes, almost four decades after its introduction, is still beset with grave difficulties. Two particularly disturbing problems are presented by insulin reactions and the unstable ("brittle") form of the disease, which seriously interfere with the pursuit of a normal life. No one can disagree with Marble,[1] who as recently as 1959 succinctly stated that "episodes of hypoglycemia due to insulin constitute one of the major problems in the treatment of diabetes."

No less of a problem is the management of unstable diabetes, a condition characterized by abrupt fluctuations of the glycemic level, which entail wide day-to-day as well as diurnal variations in the degree of glycosuria and are accompanied, as a rule, by intermittent ketosis. In 1955 Colwell[2] estimated that about 10 per cent of insulin-treated diabetics, the majority of them juveniles, fall into this category. But who can draw a sharp line of demarcation between the group of explicitly unstable patients and many others who suffer hypoglycemic reactions of variable frequency and severity? Diabetics suffering the inconveniences and ill effects of hypoglycemic reactions undoubtedly constitute a much higher quota than 10 per cent of insulin-treated patients.

In this communication I wish to call attention to the fact that unstable diabetes, in the case of most adult subjects, is not an idiopathic entity, but a direct consequence of excess insulin action.

This cause and effect relationship is clearly demonstrated in extensive, detailed laboratory data and observations which I have accumulated since 1935 and published last year.[3] These records reveal that patients treated with such doses of insulin that can cause hypoglycemic episodes show a consistent pattern of periodic ebb and tide in the extent of glycosuria, the highest glycosuric tides always occurring in the wake of frank hypoglycemic reactions; but it must be noted that *substantial flare-ups of glycosuria take place even after symptomless, mild degrees of hypoglycemia.*

Anyone who is unaware of the paradoxical fact that *hypoglycemia begets hyperglycemia* (and also ketosis)[4] —i.e., that excess insulin action exacerbates the diabetic syndrome—will be prompted to combat the hypoglycemia-induced glycosuric tide by stepping up the insulin dosage. As a consequence, hypoglycemic episodes increase in frequency and severity, and fluctuations of the blood sugar level become more abrupt and more expansive in both directions; this is then manifested in increasing diurnal variations in the urine sugar, ranging from zero to 5-6 per cent and even higher concentrations. *A vicious cycle leading to higher and higher insulin dosage ensues,* the severity of diabetes progressively increases and can advance to an extremely unstable (unmanageable) state.

This concept implies that severe states of diabetes can be reversed by the application of a regimen in which hypoglycemic episodes—even mild, symptomless degrees of it—are avoided with meticulous care. I have presented experimental proof that this is actually the case. In a paper published in 1951[5] I described an experiment demonstrating that a diabetic man, who had been consistently aglycosuric on dietary therapy alone during the preceding eleven months, developed massive glycosuria and ketosis directly after a not too severe hypoglycemic reaction induced by the intravenous injection of 7 units of insulin in the postabsorptive state. With temporary, cautious insulin therapy he again became aglycosuric without insulin. In another experiment[6] the subject was a man who, after having been an unmanageable diabetic for a number of years on 60 to 180 units of insulin, was restored to a mild state of diabetes by the avoidance of hypoglycemic states, and was readily controlled and perfectly stabilized on 16 units. When, for the experiment, the dosage was raised to 22, then to 24 units per day, it produced hypoglycemic episodes, and the patient promptly reverted to an unstable state, with the characteristic fluctuations of the blood sugar level and glycosuria, accompanied by intermittent ketosis. After this experiment the patient was once more rehabilitated to a mild state of diabetes, and again was easily controlled on only 16 units of insulin.

On the basis of hundreds of case histories in my files and those of my colleagues, it can be stated without qualification that every severe case of diabetes which came our way that had been treated for years with large doses of insulin (30-150 units), responded favorably to application of the precept that *"avoidance of hypoglycemia, even of mild, asymptomatic degrees, is no less important than the control of excessive hyper-*

glycemia and glycosuria."[6] Every one of them was restored "to a status of mild diabetes that can be satisfactorily managed with small doses of insulin or, not infrequently, without insulin." And it may be added that as a result of observing this rule not one of our newly diagnosed cases has drifted into a severe state, even if the initial condition exhibited all of the "classical symptoms" of severe diabetes and required large doses of insulin at the outset. As a matter of fact, the adult patients—with a few exceptions—eventually became aglycosuric without insulin.

The causal connection between excess insulin action and its hyperglycemic-glycosuric aftermath was uncovered in these studies in purely pragmatic procedures, with guidance by facts reaped from a wealth of laboratory tests (mostly quantitative analysis of urine samples collected in fractions around the clock, over extended periods of time). With satisfactory documentation on hand, it was matter of course to inquire into the nature of the physiologic factors and processes underlying the diabetogenic effect of hypoglycemia. We found that what we had accomplished with a purely empirical approach, was nothing more than incorporation in the application of insulin therapy of physiologic laws broadly treated in the literature in the course of the past three and a half decades. Cannon[8] was the first investigator to demonstrate that hypoglycemia—even a slight fall of the blood sugar below the postabsorptive level—stimulates the rate of release of adrenalin into the blood stream. Almost simultaneously, Houssay[7] arrived at the same conclusion by an entirely different experimental approach. Eventually, numerous workers unanimously confirmed these findings, while others subsequently showed that insulin hypoglycemia also accelerates the release of anterior pituitary and adrenal cortical hormones.

These hormones can be designated as "indirect insulin antagonists"; they do not destroy insulin, nor do they inhibit its action, but act in counter-current to insulin action and thereby cancel it. In other words: While insulin enhances assimilation of glucose (transfer into tissue cells), these antagonists simultaneously promote dissimilation. The remarkable constancy of the blood sugar level of healthy persons in the postabsorptive state reflects the sensitivity and promptness of the adrenal-pituitary system in the prevention of hypoglycemic states; it is this normal homeostatic function which is forced to operate on a greatly magnified scale, when hypoglycemia reaches unphysiologically low levels. Under this condition the overly excited glands release excessive amounts of insulin antagonists, which overshoot the goal and thus produce hyperglycemic tides. Consequently the insulin antagonists, instead of serving the homeostatic process, rudely upset and disrupt it.

This is the main pathway through which excess insulin action exacerbates the diabetic syndrome, but there are accessory elements which it brings into play. Hypoglycemic episodes, as is known, entail mental and emotional disturbances, often of severe dimensions. When Graef[5] pointed out that "severe, progressive diabetes is possibly severe and progressive because of challenges to the pituitary and adrenal glands in everyday life," he—if I understand him correctly—had reference to all environmental influences, internal as well as external. This assumption, I feel, is undoubtedly correct; but by the same token, inconveniences and emotional and mental disturbances, attendant to recurrent hypoglycemic reactions, contribute to the "downward progress" of diabetes to a greater extent than occasional incidents occurring in everyday life.

There is still another facet to the problem, and this is that hypoglycemia constitutes a stressor agent, fraught with the danger of alarm and adaptation diseases. As Guest[9] put it, "there is reason to believe that wide and abrupt fluctuations between hyper- and hypoglycemia, and periodic development of ketosis, constitute situations of stress with attendant mutually aggravating metabolic derangements that have far-reaching deleterious consequences."

The deleterious effect of excess insulin action is by no means confined to diabetic persons; it exerts a diabetogenic effect also in cases of organic hyperinsulinism produced by functioning islet cell tumors, and in the state of iatrogenic (artificial) hyperinsulinism, introduced in nondiabetic persons (mental patients or healthy individuals) by insulin injections.[10] The diabetic patient who is subject to hypoglycemic episodes obviously presents a condition in which iatrogenic hyperinsulinism is superimposed on an already deranged state of metabolism and by virtue of this fact is likely to exert its adverse effects with greater potency than in nondiabetic subjects.

From the foregoing it is evident that hypoglycemic episodes, the state of unstable diabetes, and stress and alarm reactions do not present separate problems in the insulin therapy of diabetes; the *agent provocateur* that ushers in all of these phenomena is one and the same factor: iatrogenic hyperinsulinism.

In conclusion I must sound a *serious warning against any abrupt and radical reduction of the insulin dosage.* The changes must be made gradually in a rather laborious procedure, and cannot be successfully carried

out without the judicious use of extensive quantitative laboratory tests. As I pointed out in my detailed article,[3] haphazard changes will in most cases invite grave consequences.

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MICHAEL SOMOGYI, PH.D.
St. Louis

BOOK REVIEWS

ARTERIAL EMBOLISM IN THE LIMBS. By A. L. Jacobs and C. G. Rob. $8.00, pp. 200, The Williams and Wilkins Company, Baltimore, Maryland, 1959.

This book is impressive as a sincere and objective study of the problem of arterial embolism. The author supports his conclusions by data based on dissections and injections as well as on first-hand examination of patients (eighty-one case histories are given in the Appendix). The conclusions themselves are attractively and provocatively summarized in the last chapter as a series of aphorisms, for example: "Embolism is commonest in the smallest arteries and least common in the largest. This natural anatomical incidence is not evident in hospital series because many of the less serious cases do not come under observation." "Arterial pulsation, though usually weakened, is not uncommonly felt beyond the site of an embolic occlusion in a large limb artery." "Though the number of successes is not great, embolectomy holds first place in the treatment of embolism of the larger limb arteries." Most of these seem useful, although some vascular surgeons might take exception to the following one: "In limbs with adequate collateral circulation, signs of grave ischaemia do not persist for more than two hours after onset." On the whole, this thoughtful book should prove to be extremely helpful for those concerned in the management of sudden vascular occlusions in the extremities.

PANCREATITIS. A CLINICAL-PATHOLOGIC CORRELATION. By Herman T. Blumenthal and J. G. Probstein. $9.50, pp. 379, Charles C Thomas, Springfield, Illinois, 1959.

This useful book may be described as encyclopedic within a small compass. It is remarkable that the authors have been able to condense a great mass of literature so successfully, in addition to analyzing carefully their own series of 163 cases. One consequence of covering the field so completely is that some newer developments, such as the association of pancreatitis with hyperparathyroidism and hyperlipemia, and recent studies on enzymes in the serum, are more or less submerged in the general discussion. This is perhaps of little moment, however, since the newness of such observations does not necessarily endow them with special importance.

The authors classify acute pancreatitis into etiologic types as follows: infectious, vascular, biliary obstructive, pancreatic obstructive, metabolic, toxic and chemical, traumatic, allergic, idiopathic. The last named is still the largest single category (over one third of cases in their series). The most vexatious problems in this respect are well brought out. Among the interesting points in their discussion of etiology and pathogenesis is the suggestion that some of the more recently recognized pancreatic enzymes, such as collagenase and elastase, play an important part in breaking down tissue in the acute disease.

The authors present interesting hypotheses to explain the mechanism of clinical manifestations in some cases. The propensity of acute pancreatitis for mimicking other diseases is indicated. In considering treatment, they emphasize that the management of the acute phase is primarily medical, while the treatment of the complications and sequelae is largely surgical. The importance of watching closely for loss of blood volume and of electrolytes is properly emphasized. The management of disturbed carbohydrate metabolism is considered briefly, with a note of caution about the use of insulin, and consideration of the effect of hypoglycemia on the secretory activity of the exocrine pancreas.

A minor criticism may be leveled at the index, where many page references are listed under a given subject, with no indication as to which one represents the major discussion of the problem. As a consequence, a good deal of thumbing of pages is required to find certain information. In a future edition bold-face type might be used to indicate relative importance.

This volume seems certain to become a standard reference work in a difficult and obscure field.

BOOKS RECEIVED

DIABETES MELLITUS. Third Congress of the International Diabetes Federation, Düsseldorf, July 21-25, 1958. Edited by Prof. Dr. K. Oberdisse and Priv.-Doz. Dr. K. Jahnke, Düsseldorf. German, English and French summaries of each paper are included. $41.65, pp. 799, 225 partly colored illustrations, 178 tables, 1959. Georg Thieme Verlag, Stuttgart. In the U.S.A. and Canada, Intercontinental Medical Book Corporation, New York 16, New York.

Lester J. Palmer
1890-1959

Blair Holcomb, M.D., Portland, Oregon

With the death of Dr. Lester J. Palmer of Seattle on Nov. 29, 1959, the diabetic patients of the Pacific Northwest lost a valued counselor and friend and the American Diabetes Association a dedicated and devoted member and worker.

Dr. Palmer, a native of Kansas, received his M.D. degree at Northwestern Medical School, interned at Cook County Hospital, Chicago, and served a residency at St. Luke's Hospital in Chicago. Arriving in Seattle, he helped found the Virginia Mason Clinic. In 1922 he began to show active interest in diabetes and early saw the need for education of the patient. To this end he instituted a class instruction program for his patients which he personally conducted, inspiring them with his enthusiasm and interest.

Within a few years, his growing number of patients and friends helped him to organize The Diabetic Trust Fund. Through this fund many activities for the welfare of diabetic patients, particularly the operation of summer camps for diabetic children, were financed. To these camps he gave unselfishly of himself, visiting the two camps, one on Vashon Island and the other on Hoods Canal, several times each summer. Concrete evidence of his continued interest is his bequest to The Diabetic Trust Fund. Also, in his memory, we are told, were many gifts from his patients and friends to the same fund.

Impressed by the necessity of maintaining continuity in education of the patient he established *The Diabetes Monthly,* now in its twenty-third year, sending it to his patients throughout the country.

He was a pioneer in the founding of The Washington Diabetes Association, and as its president for many years acted as a spark plug to maintain the interest of both lay and professional members.

In addition to these activities in the field of diabetes, he found time somehow to act as Chief of the Department of Medicine of the Virginia Mason Clinic and President of the Board of Trustees of The Virginia Mason Hospital and Chief of Staff of the same hospital for many years. In medical education he was Clinical Professor of Medicine of the University of Washington Medical School. In his spare moments, he was secretary of the North Pacific Society of Internal Medicine from its inception and finally its president. It should go without saying that he was a member of the American College of Physicians, The American Medical Association and the King County Medical Society.

Nor was his life devoted exclusively to medicine. He took his civic responsibilities seriously, and was an active member of many organizations.

One may well wonder how in such a busy life he had the vitality to engage intensively in the work of the American Diabetes Association. Our older members will recall that he became a member of our organization

in 1941, was elected to the Council in 1944, and served ten years. He was installed as President in 1950 in which office he served with great distinction.

In the first four years on the Council he was Chairman of the Committee on Insulin Syringe Unification, bringing order out of chaos. In 1951, he was made Chairman of the Committee on Affiliate Associations, later Vice Chairman, making good use of his background information and wisdom in helping to solve the complex problems of this phase of our organization. He also was a working member of many other committees too numerous to mention.

Early in 1959, previously in reasonably good health, he learned that he had developed a bronchogenic carcinoma. Intensive cobalt therapy, apparently successful, resulted in pneumonitis and, as a terminal event, congestive cardiac failure. Not over two weeks before his death, I visited for an hour with him at his home. I shall always be happy to recall that even at that late date, he mustered enough fire and enthusiasm to reminisce about our experiences together through the years, our arguments and agreements, but most of all, about the story of the American Diabetes Association.

Dr. Palmer was devoted to his family. He is survived by his wife, his son, and one grandson. Indeed, we shall all miss Lester Palmer.

ABSTRACTS

Abraham, S.; Cady, P.; and Chaikoff, I. L. (Dept. of Physiol., Univ. of California, Berkeley, Calif.): GLUCOSE AND ACETATE METABOLISM AND LIPOGENESIS IN MAMMARY GLANDS OF HYPOPHYSECTOMIZED RATS IN WHICH LACTATION WAS HORMONALLY INDUCED. Endocrinology 66:280-88, February 1960.

Lactation was maintained by the daily administration of mammotrophin, Δ^1 hydrocortisone acetate and somatotrophin in rats hypophysectomized in midpregnancy. The omission of somatotrophin did not interfere with lactation but the omission of mammotrophin did. Any of the three hormones alone failed to sustain lactation. The conversion of glucose carbons 1,2, and 6 to CO_2 and fatty acids and the stimulating effect of glucose on lipogenesis from acetate was studied. The normal lactating mammary glands and the hormonally maintained one demonstrated a metabolic pattern implying a non-Embden Meyerhof pathway in the conversion of glucose to fatty acids. The nonlactating but enlarged glands of (1) rats not receiving daily postpartum hormone injection and (2) rats receiving daily postpartum injections which were stopped four days before study revealed no significant participation of the hexose-monophosphate shunt in the conversion of glucose to fatty acids. Increased activity of this pathway was related to milk formation and secretion and not glandular growth and development. H.L.W.

Baldwin, R. S.; Griese, G. G.; Heersma, J. R.; and Lewis, R. F. (Marshfield, Wis.): DIABETES IN PREGNANCY. GP 19:109-15, June 1959.

Forty-six pregnant diabetic women were seen over a twenty-year period in Marshfield, Wisconsin. About 20 per cent of the ninety-one pregnancies were unsuccessful. Survival rate of the remaining seventy-four pregnancies was 71 per cent. There were twelve neonatal deaths. Severity and duration of the mother's diabetes were related to the outcome. Diagnosis of diabetes and management of pregnancy are discussed. Hospitalization should be considered when the patient reaches thirty to thirty-four weeks. Use of hormones for therapy is of doubtful value. Lente mixtures were used frequently. Primigravidas with severe diabetes are delivered by cesarean section. Multiple doses of Regular Insulin are given before delivery. Conventional neonatal care is advised. A.R.C., JR.

Bally, Peter R.; Cahill, George F.; Leboeuf, Bernard; and Renold, Albert E. (Dept. of Medicine, Harvard Medical School and Peter Bent Brigham Hosp., and Baker Clin. Res. Lab., New England Deaconess Hosp., Boston, Mass.): STUDIES ON RAT ADIPOSE TISSUE IN VITRO. V. EFFECTS OF GLUCOSE AND INSULIN ON THE METABOLISM OF PALMITATE-1-C^{14}. J. Biol. Chem. 235:333-36, February 1960.

Rat adipose tissue was capable of incorporating palmitate-1-C^{14} bound to albumin. The effect was increased by glucose alone or insulin in the presence of glucose. It is suggested that in the absence of glucose production of glycerol, phosphate limits the incorporation of fatty acids by adipose tissue.

A.R.C., JR.

Barta, L. (Kinderklinik, Universität Budapest, Hungary): ON THE QUESTION OF THE HEREDITY OF DIABETES MELLITUS. Deutsche med. Wchnschr. 85:558-59, April 1, 1960.

The author evaluated the familial incidence of diabetes mellitus in three groups of pediatric patients. Group I consisted of the families of 227 consecutive cases of juvenile diabetes; Group II of the families of 251 obese but nondiabetic children, and Group III of the families of 900 children who had neither signs nor symptoms of any endocrine disturbance. In this latter control group, eighteen siblings with diabetes were detected, an incidence of 2 per cent, which according to the author corresponds with the over-all incidence of diabetes in Hungary. The obese children had fourteen diabetic siblings (an incidence of 5.5 per cent), the diabetic children thirty-seven or 15.4 per cent diabetic siblings. A surprising result was found when the author analyzed the incidence of the sibling diabetes among the parents, the grandparents and more distant relatives. The parents of the children without any endocrinopathy were entirely free of diabetes, with the disease occurring only in the grandparent generation or among more distant relatives. The incidence of parental diabetes was, how-

ever, almost identical in the groups of the diabetic and the obese children (2.7 per cent and 2.8 per cent). On the other hand, the diabetic children had about four times as many diabetic grandparents as the obese or the normal control children (6.6 per cent vs. 1.6 per cent and 1.4 per cent). The author, attempting to explain the relatively high incidence of parental diabetes among the obese children, suggests that obesity is common among parents of obese children. Obesity may contribute to the manifestation of diabetes in adulthood, but does not affect the carbohydrate tolerance in childhood—a rather unconvincing argument. (German) M.G.G.

Beatty, Clarissa H.; Peterson, Ruth D.; and Bocek, Rose Mary (Dept. of Biochemistry, Univ. of Oregon Med. Sch., Portland, Oreg.): EFFECT OF INSULIN ON CARBOHYDRATE METABOLISM OF SKELETAL MUSCLE FIBERS AND DIAPHRAGM FROM CONTROL AND PANCREATECTOMIZED RATS. J. Biol. Chem. *235*: 277-81, February 1960.

A method utilizing viable rat adductor muscle groups in a Warburg apparatus to estimate metabolism has certain advantages over the rat diaphragm technic. Glucose uptake, glycogen synthesis and carbon dioxide or lactate production were similar for the two types of muscle. Glucose uptake was diminished in muscle from depancreatized rats, and this was corrected by addition of insulin. Lactate accumulation was increased in muscle from diabetic animals but there was no change in glycogen content or oxygen consumption. A.R.C., JR.

Beck, John C. (McGill University Clinic, Royal Victoria Hosp., Montreal, Que.): PRIMATE GROWTH HORMONE STUDIES IN MAN (SUMMARY). Proc. Roy. Soc. Med. *52*:1047-48, December 1959.

Human growth hormone (HGH) was injected intramuscularly into nine human subjects during metabolic balance studies. Blood urea nitrogen and serum nonprotein nitrogen had fallen significantly on the second day of HGH injection (5 to 20 mg. per day). These effects lasted two to six days after discontinuance of HGH. Prompt nitrogen and potassium retention, maximal by the third to sixth day of treatment, persisted three to twelve days after stopping HGH. Calcium and phosphorus retention persist after stopping HGH even beyond this time. No significant change in serum concentration of sodium, potassium, chloride, bicarbonate, calcium, cholesterol, cholesterol esters, total lipids, total fatty acids, lipid phosphorus, mucoproteins or glucosamine was observed during HGH administration.

HGH in doses ranging from 200 to 600 μg. per kg. body weight per day in hypopituitary subjects consistently led to impaired carbohydrate tolerance. A single intramuscular injection of 5 mg. of HGH produced an initial fall in plasma nonesterified fatty acids most marked after twenty to thirty minutes and followed by a maximal rise at four to six hours later. Administration of HGH to a fifteen-year-old pituitary dwarf over a nine-month period has resulted in an increase in both height and body weight. G.A.W.

Bessman, Samuel P. (Dept. of Pediatrics, Univ. of Maryland Sch. of Med., Baltimore, Md.): DIABETES MELLITUS: OBSERVATIONS, THEORETICAL AND PRACTICAL. J. Pediat. *56*: 191-203, February 1960.

The author reviews some aspects of diabetes mellitus in the light of present knowledge. The change in the natural history of the disease is discussed. Earlier diagnosis made by frequent testing of individuals with a family history has meant that new cases more often present with only mild symptoms and only rarely with coma. Hypoglycemia is mentioned as an occasional early manifestation.

The fluctuating insulin requirement in early diabetes is discussed, and possible causes are mentioned. It is thought possible that the early sensitivity to insulin might be related to disease of the liver and adrenal cortex, reducing the ability of these organs to combat the hypoglycemic effect of insulin, producing hypoglycemia much the same as is seen in liver disease and Addison's disease. An interesting theory is presented explaining the conflicting reports from various clinics about the relationship between degree of control and incidence of complications. It is suggested that by a process of selection patients who are difficult to control gravitate to those physicians who do not insist on careful control, while those doctors who insist on careful control have a higher percentage of easily controlled people.

In discussing the mechanism of action of insulin and the defect in diabetes the author cites evidence to show that the defect in carbohydrate metabolism is only one of several defects in the diabetic patient. Evidence also is mentioned which is not in keeping with the theory that the sole site of action of insulin is at the cell membrane.

The therapeutic implications of this knowledge are discussed from the standpoint of the oral hypoglycemic agents. R.L.J.

Bischoff, A. (Neurological Clinic, University of Zurich, Switzerland): ABOUT DIABETIC AMYOTROPHY (NEUROMYOPATHY). Schweiz. med. Wchnschr. *89*:519-25, May 16, 1959.

Amongst fifty patients with diabetic neuropathy the author observed twelve with diabetic amyotrophy as described by Garland (1955). This syndrome is characterized by motor palsies of the proximal muscles of the lower extremities and in the pelvic region, mainly of the quadratus femoris. In three cases shoulder musculature was also involved. All patients were older than fifty years. In two, the diabetes was latent, recognizable only on glucose tolerance tests. Electromyographic tracings are discussed. Muscle biopsies showed a mixture of normal and atrophic muscle fibers. The literature on this subject is extensively and ably discussed. (German) G.R.C.

Bojesen, Ejgil; and Egense, Johan (Inst. of Experimental Medicine, Division of Endocrinology & Inst. of Med. Physiol., Copenhagen, Denmark): ELIMINATION OF ENDOGENOUS CORTICOSTEROIDS IN VIVO: THE EFFECTS OF HEPATECTOMY AND TOTAL ABDOMINAL EVISCERATION IN THE ACUTELY ADRENALECTOMIZED CAT, AND THE EFFECT OF MUSCULAR EXERCISE AND INSULIN ADMINISTRATION ON THE ISOLATED HINDQUARTER PREPARATION. Acta endocrinol. *33*:347-69, March 1960.

The rate of elimination of endogenous steroids, cortisol and corticosterone, was measured in anesthetized, acutely adrenalectomized cats. The blood level was measured and the rate of elimination was evaluated before and after elimination of liver and other intra-abdominal organs. It was concluded that the main area of catabolism was in the liver and intestinal tract, with almost equal activity in each.

The hindquarter used to evaluate the role of skeletal muscles in this process showed very little elimination; and no enhancement resulted from either electrical stimulation or administration of large doses of insulin locally. S.B.B.

Brandman, Otto; and Rifield, Lewis (Harrison S. Martland Med. Center and Seton Hall College of Med. and Dentistry, Post-Graduate Dept., Newark, N. J.): CHLORPROPAMIDE IN

DIABETES, ONE YEAR'S EXPERIENCE. Acad. Med. New Jersey Bull. 5:119-28, December 1959.

Forty-eight patients, all over forty, were placed on chlorpropamide for a period of at least one year. Laboratory studies on all patients included a complete blood count, platelet count, blood sugar, urea nitrogen, cephalin flocculation, thymol turbidity and alkaline phosphatase determination. These tests were repeated at weekly intervals for four weeks, every other week for the next month, and monthly thereafter. In a moderate percentage of cases there appeared to be an hepatic dysfunction. No influence on the red or white blood cell count or platelets was noted. Chlorpropamide seemed to be a more effective hypoglycemic agent than tolbutamide, capable of producing definite hypoglycemic reactions. W.R.K.

Cooper, Gerald R.; Quinlan, Carroll B.; Whitner, Virginia S.; and Barrow, J. Gordon (Communicable Disease Center and Heart Disease Control Program, Public Health Service, U. S. Dept. of Health, Education & Welfare; Heart Disease Control Program, Georgia Dept. of Public Health; Dept. of Med., Emory University Sch. of Med.; and Grady Memorial Hosp., Atlanta, Ga.): ATHEROGENESIS AND ITS RELATION TO DIABETES. J.M.A. Georgia 49:99-105, March 1960.

Very little information is available about therapeutic measures designed to control atherogenesis in diabetic individuals. Many physicians feel that the best therapy for control of excessive atherogenesis in diabetes is to keep the patient in good metabolic control, maintain well-being, and keep physical and mental stress at a minimum. The impression prevails that the frequency of atherosclerosis in diabetic patients who have never suffered from diabetic acidosis and who follow their control regimen, is no greater than that in healthy persons. The suggestion has been made that not only should the diabetes be kept in good control, but it is important to maintain in a normal range both: (1) the blood pressure, to counteract local vascular factors, and (2) the serum lipids, to minimize the plasma factors suspected of enhancing atherogenesis. No evidence suggests that additional measures beyond those reported useful for control of atherogenesis in nondiabetics, are indicated in the preventive therapy of atherogenesis in diabetic patients. W.R.K.

Dekaban, Anatole; and Baird, Robert (Section on Developmental Neurology, National Institute of Neurological Diseases and Blindness, National Institutes of Health, Public Health Service, Bethesda, Md.): THE OUTCOME OF PREGNANCY IN DIABETIC WOMEN. I. FETAL WASTAGE, MORTALITY, AND MORBIDITY IN THE OFFSPRING OF DIABETIC AND NORMAL CONTROL MOTHERS. J. Pediat. 55:563-76, November 1959.

The outcome of 235 pregnancies in diabetic and prediabetic women was investigated and the results obtained were compared with the corresponding data in the 249 pregnancies of matched normal controls. The percentage of abortions and previable deaths in the diabetic sample was 29.9, in the prediabetic sample 20.5, and in the normal controls 12.4. Stillbirths occurred with the following frequency: 11.5 per cent in the sample of diabetic mothers, 5.1 per cent in the prediabetics, and 1.2 per cent in the normal controls. The neonatal deaths amounted to 8.3 per cent of all pregnancies in the diabetic women, 1.3 per cent in the prediabetics, and 3.6 per cent in the sample of normal controls. These values demonstrate clearly that the fetal wastage in the diabetic women was significantly greater than in the normal controls. The fetal loss during the prediabetic stage was about midway between that in diabetic and normal control mothers. The results obtained generally confirm the findings of other investigators. The main purpose of this study was to test the hypothesis that the morbidity dating since birth in the offspring of diabetic mothers is also increased as compared with the sample of normal controls. There were six abnormal surviving offspring in the sample of 157 diabetic pregnancies, which amounts to a total of 3.8 per cent (or 7.6 per cent of the surviving children). In the normal control sample of 249 pregnancies there was only one abnormal surviving offspring, an incidence of 0.4 per cent. The difference in percentages in these two groups was significant at P less than 0.01, indicating that the hypothesis is very likely correct. The abnormalities in these children include mental deficiency, congenital malformations, birth injury, and epilepsy. W.R.K.

Delahunt, Charles S.; P'an, S. Y.; Dardin, Vincent J.; and Schneider, Jurg A. (Dept. of Macrobiology, Res. Lab., Chas. Pfizer & Co., Inc., Groton, Conn., and Dept. of Pathology, Georgetown Med. Center, Washington, D.C.): PROLONGED ADMINISTRATION STUDIES WITH CHLORPROPAMIDE. Toxicol. & Appl. Pharmacol. 2:195-205, March 1960.

In prolonged feeding studies extending more than two years, rats consumed chlorpropamide in quantities up to 500 mg./kg. without evidencing any hematologic or histologic aberrations. Drug levels of 250 mg./kg. and above did cause a decrease in growth rate. A canine oral tolerance study conducted for more than a year at doses up to 150 mg./kg. per day did not produce any adverse effects on biochemical, hematologic, gross anatomical, or microscopic findings. Repeated oral administrations to monkeys for eleven months did not cause any abnormalities. W.R.K.

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de Roetth, Andrew, Jr.; and Pei, Yen Fen (Dept. of Ophthalmology, Columbia University, Coll. of Physicians and Surgeons, and the Inst. of Ophthalmology, Presbyterian Hosp., New York, N. Y.): EXPERIMENTAL DIABETIC RETINOPATHY: RETINAL METABOLISM IN THE ALLOXAN DIABETIC RAT. A.M.A. Arch. Ophth. 63:226-31, February 1960.

Alloxanized rats were killed at monthly intervals, their retinas removed and placed in Warburg vessels. Oxygen utilized by retinas from diabetic animals was increased when compared to normal consumption, and it was greater when pyruvate served as the substrate instead of glucose. Anaerobic glycolysis was reduced by alloxan diabetes. Retinopathy will be studied further by prolonging survival of the animals with insulin. A.R.C., JR.

Dolger, Henry (Dept. of Medicine, The Mount Sinai Hosp., New York, N. Y.): AN ASSESSMENT OF ORAL ANTIDIABETIC THERAPY. J. Mt. Sinai Hosp. New York 27:33-39, January-February 1960.

When tolbutamide became an established form of oral diabetes therapy, it had to measure up to insulin as a standard for therapeutic comparison. The generally satisfactory experience with tolbutamide now makes this drug the standard for comparison by which all other modifications of the original sulfonylurea formula and unrelated compounds must be measured. W.R.K.

Egeli, Ekrem Serif; and Berkmen, Rasim (Third Clinic of Internal Medicine, University of Istanbul, Istanbul, Turkey): ACTION OF HYPOGLYCEMIA ON CORONARY INSUFFICIENCY AND MECHANISM OF ECG ALTERATIONS. Am. Heart J. 59: 527-40, April 1960.

Hypoglycemia was produced by injection of 0.2 U. of insulin

per kilogram of body weight in thirty-eight patients with severe coronary arteriosclerosis (with angina or previous myocardial infarction). Angina pectoris was not produced but ECG changes resulted. The occurrence of the latter were better correlated with the decrease in blood potassium than blood sugar. They were prevented or reversed by administration of potassium.

Further experiments with epinephrine and regitine in the same group served to strengthen the evidence that the decrease in blood potassium rather than blood sugar was responsible for the ECG alterations. S.B.B.

Eliasson, Sven G.; and Hughes, Ann H. (Dept. of Internal Medicine, University of Texas Southwestern Med. Sch., Dallas, Tex.): CHOLESTEROL AND FATTY ACID SYNTHESIS IN DIABETIC NERVE AND SPINAL CORD. Neurology *10*:143-47, February 1960.

The incorporation of acetate-1-C^{14} into fatty acids and cholesterol of spinal cord and nerves was studied. Normal rats and alloxan diabetic rats were used. In comparison with normal values, cholesterol synthesis is depressed in diabetic spinal cord and nerve. Fatty acid synthesis is depressed in diabetic cord and elevated in diabetic peripheral nerve. Pyridine nucleotides stimulate lipogenesis and cholesterol synthesis in normal cord and nerve but do not influence the depressed production in the diabetic animal. W.R.K.

Freinkel, Norbert; and Goodner, Charles J. (Thorndike Memorial Lab. and Second and Fourth (Harvard) Med. Serv. Boston City Hosp., and the Dept. of Medicine, Harvard Med. Sch., Boston, Mass. & the Howard Hughes Med. Inst.): CARBOHYDRATE METABOLISM IN PREGNANCY, I. THE METABOLISM OF INSULIN BY HUMAN PLACENTAL TISSUE. J. Clin. Invest. *39*:116-31, January 1960.

The human term placenta was analyzed for the presence of a system for the proteolytic inactivation of insulin. The insulin used was the I^{131}-tagged compound and an inactivating system was found resembling that described in animal liver in most physical and chemical characteristics. The author discussed the probable role that such an inactivating system might play in the increased insulin requirements of the diabetic pregnancy and decreased effectiveness of injected insulin of normal pregnancy. S.B.B.

Gastineau, Clifford F.; Power, Marschelle H.; and Rosevear, John W. (Mayo Clinic, Rochester, Minn.): METABOLIC STUDIES OF A PATIENT WITH OSTEOPOROSIS AND DIABETES MELLITUS: EFFECTS OF TESTOSTERONE ENANTHATE AND STRONTIUM LACTATE. Proc. Staff Meet. Mayo Clin. *35*:105-11, March 2, 1960.

A thirty-five-year old man with diabetes and severe osteoporosis has been studied with calcium balance technics while under treatment with testosterone enanthate and subsequently with testosterone enanthate plus strontium lactate. The use of testosterone enanthate alone was followed by a return of a previously negative calcium balance toward normal, but only after the addition of strontium lactate was a positive calcium (actually, calcium-plus-strontium) balance achieved. There was continued clinical improvement during both programs of treatment, but it cannot be said how much of the symptomatic betterment was the result of these agents and how much was the consequence of better protection of the subject's skeleton against stress. W.R.K.

Givner, Isadore (108 East 66th St., New York 21, N. Y.): JUVENILE DIABETES. Am. J. Ophth. *49*:358-60, February 1960.

At Camp NYDA, sponsored by the New York Diabetes Association for diabetic children, a study of 1,000 eyes of juvenile diabetics was made for a period of twelve years (1946-1958). Cohn studied the beta-lipoproteins in 600 diabetic children. Lax carried out vasculograms to study early evidence of changes in tonus in the arterial wall. In none of the children was arteriosclerosis observed ophthalmoscopically. The changes detailed in the vasculograms are also found in arteriosclerosis, yet one cannot say that these changes exclude mere rigidity as opposed to suppleness, for we do not know what molecular changes are responsible for these peripheral changes. Patients with diseased vessels frequently show hemorrhage after hypoglycemic reactions. Every effort should be made to avoid this by careful observation, diet, and regulated exercise. In the literature some excellent results have been reported with the use of Kempner's rice diet. If one can get a patient to adhere to this diet, it is certainly worth a trial. A study of all patients with remissions in diabetic retinopathy, whether it seems spontaneous or related to physical or dietary factors, should be carefully scrutinized in hope that some common denominator shows itself. At the same time, search should be continued for a venous and capillary toxin associated with diabetes that might be neutralized or held in check. W.R.K.

Goessner, W.; and Korting, G. W. (Hautklinik und Pathologische Institut, Universität Tübingen, Germany): METASTASIZING ISLET CELL CARCINOMA OF A-CELL TYPE IN A CASE OF PEMPHIGUS FOLLIACEUS WITH RENAL GLYCOSURIA. Deutsche med. Wchnschr. *85*:434-37, March 11, 1960.

Marked glycosuria without hyperglycemia after ACTH administration was observed in a fifty-one-year-old man with pemphigus folliaceus. The renal glycosuria could be controlled by the administration of acetazoleamid (Diamox), but the patient's condition deteriorated, the pemphigus turned into the bullous type and extended, finally causing exitus in circulatory failure. At the autopsy, a malignant tumor of the pancreas was found with metastases to the liver. Histologically, an islet cell tumor could be identified, with all the staining characteristics of an α-cell carcinoma. Histochemically, the tumor cells showed marked esterase activity, considerable tryptophan content, and absence of acid phosphatase, all of which have been reported in α-cells of the normal pancreatic islets of man. The high tryptophan content of the tumor suggested the presence of glucagon which is rich in this amino acid, although a positive tryptophan reaction may occur also with serotonin and a few other substances.

A tumor extract was prepared and tested on the blood sugar of the fasting rabbit, causing marked hyperglycemia after forty-five minutes with return to normoglycemia after two hours. Since serotonin has no effect on the blood sugar, the authors assume that they dealt with a functioning α-cell tumor producing glucagon. The possible relationship of this tumor to the pemphigus is not discussed, but it is mentioned that occasionally—though rarely—pemphigus is associated with visceral malignancies. (German) M.G.G.

Greiss, Frank C., Jr. (Dept. of Obstetrics and Gynecology of the Bowman Gray Sch. of Med. of Wake Forest Coll., and the North Carolina Baptist Hosp., Winston-Salem, N. C.): PREGNANCY COMPLICATED BY DIABETES MELLITUS AND SUPERIMPOSED THYROTOXICOSIS. Obst. & Gynec. *15*:382-86, March 1960.

An unusual case of thyrotoxicosis and diabetes mellitus com-

plicating pregnancy is presented. The effects of thyrotoxicosis on diabetes, normal thyroid metabolism in pregnancy, and the effects of altered thyroid function on pregnancy and the developing fetus have been discussed. It is suggested that antithyroid therapy should be governed by clinical evaluation of the pregnant patient. Supplemental thyroxine does not necessarily protect the fetus from hypothyroidism in the presence of antithyroid drugs. Avoidance of even transient maternal hypothyroidism is mandatory. Maintenance of the patient in a subclinical thyrotoxic state is preferable. W.R.K.

Hall, James C. (Newark College of Arts and Sciences, Rutgers, The State University, Newark, N. J.): THE EFFECT OF INSULIN ON INTACT MUSCLE FROM NORMAL AND ALLOXAN-DIABETIC RATS. J. Biol. Chem. *235*:6-8, January 1960.

Production of alloxan diabetes decreased the oxygen consumption of rat skeletal, diaphragm and cardiac muscle below that of normal rat muscle. This was improved by addition of insulin, citrate or lactate except that lactate was ineffective in skeletal muscle. Glycogen content of muscle was decreased by aerobic incubation, and this effect was limited by addition of citrate or lactate and insulin except in the case of heart muscle. Insulin seems to increase the activity of the Krebs tricarboxylic acid cycle. A.R.C., JR.

Hansen, O. (Biological Labs. of Mecodumex, Dumex, Ltd. [formerly Medicinalco, Ltd.], Copenhagen, Denmark): A MICROMETHOD FOR SIMULTANEOUS DETERMINATION OF GLUCOSE AND KETONE BODIES IN BLOOD AND GLYCOGEN AND KETONE BODIES IN LIVER. Scandinav. J. Clin. & Lab. Invest. *12*:18-24, 1960.

The glucose-anthrone method is combined with a previously published (Hansen 1959) micromethod for determination of ketone bodies. The method presented allows rapid and highly specific simultaneous microdetermination of glucose and ketone bodies in blood and glycogen and ketone bodies in liver. It is possible to determine with accuracy glucose values down to 40 mg./100 ml. and ketone body values down to 0.2 mg./100 ml. The method is sensitive down to values of half these magnitudes. W.R.K.

Hardwick, Christopher; Butterfield, W. J. H.; Fry, I. Kelsey; and Briggs, J. H. (Guy's Hosp., London, S.E.1, England): DIRECT MEASUREMENT OF THE EFFECT OF INSULIN ON THE UPTAKE OF GLUCOSE BY PERIPHERAL MUSCLES IN NORMAL SUBJECTS, DIABETICS AND ACROMEGALICS. Proc. Roy. Soc. Med. *52*:807-09, October 1959.

Small doses (0.03-0.1 units) of glucagon-free insulin were made to reach the forearm tissues following injection into the brachial artery. Arteriovenous differences in the blood sugar concentration were measured from vessels supplying and draining these tissues, and a venous occlusion plethysmograph was used to determine forearm blood flow. In normal subjects, the glucose uptake, initially low before the intra-arterial insulin, rose through a maximum at about twenty minutes after insulin injection. In newly diagnosed diabetics, the glucose uptake after insulin was well above normal, while in diabetics who had been treated with insulin for several years it was subnormal at sixteen hours after the last therapeutic dose of soluble insulin. One diabetic subject known to be insulin-resistant, showed no detectable response to the test. Seven out of eight nondiabetic acromegalic subjects were very insensitive to the test. Preliminary tests with I^{131}-labeled insulin point to inability of the tissues of acromegalic subjects to accept insulin from the extracellular fluids. G.A.W.

Hartmann, Alexis F., Sr.; Wohltmann, Hulda J.; Holowach, Jean; and Caldwell, Bettye M. (Dept. of Pediatrics, Washington U. School of Medicine & the St. Louis Children's Hosp., St. Louis, Mo.): STUDIES IN HYPOGLYCEMIA. J. Pediat. *56*: 211-33, February 1960.

A twenty-two-month-old child is described who presented with a three-month history of convulsions. Low cerebrospinal fluid glucose on two occasions prompted an investigation of hypoglycemia as a possible cause of these attacks. Glucose tolerance tests were abnormal, suggesting hyperinsulinism, and insulin sensitivity tests suggested insulin resistance. Attempts to control symptoms with ACTH and steroids were unsuccessful. Laparotomy revealed an adenoma in the pancreas which was removed, effecting a complete cure. Histologic studies of the specimen are described.

Two other cases of leucine sensitive hypoglycemia are presented with results of therapy by low-leucine diets. Detailed laboratory studies are given for these patients. Both were sensitive to leucine and insulin, and the studies suggested that the hypoglycemia following leucine administration might be due to a normal rate of glucose utilization but failure of replacement of glucose from protein or glycogen sources. R.L.J.

Hernberg, C. A. (Med. Dept. of Maria Hosp., Helsinki, Finland): THE EFFECT OF HUMAN GROWTH HORMONE ON SEVERE JUVENILE DIABETES AFTER HYPOPHYSECTOMY. Acta Endocrinol. *33*:559-68, April 1960.

Growth hormone prepared from human pituitary glands was administered to humans, usually in multiple injections. This had no effect upon blood sugar in the normal subject and slight rise in blood sugar in an adult diabetic patient. There was no effect on a hypophysectomized nondiabetic and slight hyperglycemia in a patient with primary panhypopituitarism. In four juvenile diabetics who had been hypophysectomized a single injection had a striking effect in raising blood and urine sugar and causing acetonuria within twelve hours. The effect lasted from three to five days with an increase in insulin requirements and a new retinal hemorrhage in one. S.B.B.

Ingle, David J. (Ben May Lab. for Cancer Research, Univ. of Chicago, Chicago, Ill.): SUPPRESSION OF STEROID DIABETES BY WALKER CARCINOMA IN THE RAT. Endocrinology *66*:289-94, February 1960.

Steroid diabetes was induced in tube-fed rats by injection of cortisone acetate. A decline in glycosuria occurred with the growth of implanted Walker carcinoma 256. Excision of skin from rats with steroid diabetes also caused a fall in glycosuria. It is suggested by these findings and others that: (1) the increased glucocorticoids produced in response to stress answer a need of the organism and do not result in hypercorticalism, and (2) that stress may suppress a secondary manifestation of hypercorticalism (i.e. glycosuria). H.L.W.

Jakobsen, L. Kramer (Central Lab. of Alborg County Hosp., Denmark): QUANTITATIVE DETERMINATION OF BLOOD GLUCOSE USING GLUCOSE OXIDASE AND PEROXIDASE. Scandinav. J. Clin. & Lab. Invest. *12*:76-79, 1960.

An enzymatic method for the determination of blood glucose with few operative steps is described. Perchloric acid is used for deproteinization and has the advantage that it prevents precipitation of the colloidal solution of O-dianisidine formed during color development. The use of two different standard solutions of glucose serves to eliminate slight variations from the Beer-Lambert law. The glucose content of deproteinized

blood is stable for at least four hours. The enzymatic reaction should be carried out at room temperature, but in emergency cases determination can be done by incubating the test tubes for twenty minutes at 37° C. with only a slight loss of accuracy. The content of nonfermentable reducing substances corresponds to 10-15 mg./100 ml. glucose, when the total reducing power of blood is determined by the Hagedorn, Halstrøm and Norman Jensen method. During glucose tolerance tests the amount of nonfermentable reducing substances undergoes variation. W.R.K.

Lennon, E. J.; Engbring, N. H.; and Engstrom, W. W. (Dept. of Medicine, Marquette University Sch. of Medicine, and Milwaukee County Hosp., Milwaukee, Wis.): EXPERIENCES WITH THE USE OF ORAL HYPOGLYCEMIC AGENTS IN DIABETES MELLITUS. Wisconsin M. J. 59:191-96, March 1960.

Of 676 diabetic patients under observation in a diabetes clinic, 104 patients were treated with tolbutamide for periods of two to thirty-two months. The degree of response was defined and in these terms, 76 per cent of the patients had "good" to "excellent" control while receiving this agent. All patients were selected. Mild adverse reactions occurred in seven patients, but in four of these, symptoms disappeared as the drug was continued. Although in general the middle-aged, moderately obese, "nonketotic" diabetic patient who had had diabetes for ten or less years and who was controlled by thirty or less units of insulin daily seemed most apt to have a favorable response; none of the usual criteria for predicting success or failure could be invariably applied to individual cases. Weight gain frequently occurs in those patients managed with the arylsulfonylureas. It is wise to consider this in selecting patients, continuing dietary management alone in the patient who is already markedly obese. In some cases, "secondary failure" of tolbutamide therapy seemed to be associated with deviations from diet and weight gain. W.R.K.

Mach, Bernard; Field, R. S.; and Taft, E. B. (Harvard Med. Sch., Boston, Mass.): METAHEXAMIDE JAUNDICE. New England J. Med. 261:438-40, Aug. 27, 1959.

A case of jaundice after administration of metahexamide is presented. Laboratory and histologic data revealed that the lesion was primarily cellular and resembled that of a mild hepatitis as seen in iproniazid toxicity. S.S.

Madison, Leonard L.; Combes, Burton; Adams, Reuben; and Strickland, William (Dept. of Internal Med., Obstetrics and Gynecology, and Surgery, University of Texas Southwestern Med. Sch., Dallas, Tex.): THE PHYSIOLOGICAL SIGNIFICANCE OF THE SECRETION OF ENDOGENOUS INSULIN INTO THE PORTAL CIRCULATION III. EVIDENCE FOR A DIRECT IMMEDIATE EFFECT OF INSULIN ON THE BALANCE OF GLUCOSE ACROSS THE LIVER. J. Clin. Invest. 39:507-22, March 1960.

An attempt was made to determine the effect of insulin on hepatic output of glucose in dogs. This was performed on twenty-four dogs with end-to-side portacaval shunts in order to exclude the splanchnic bed from the system. In addition, the insulin was infused slowly to avoid counterregulatory effects of hypoglycemia. A striking decrease in hepatic output was demonstrated by this method within the first few minutes up to sixty minutes after intravenous injection of insulin. As predicted, a rapid injection of insulin with production of hypoglycemia prevented such a decrease and simultaneous infusion of glucose potentiated the result. S.B.B.

Moeller, J.; and Lohse, F. (Dept. of Medicine, University of Würzburg, Germany): THE EFFECT OF PROLONGED GLUCOSE LOAD ON THE NORMAL RENAL THRESHOLD FOR GLUCOSE. Klin. Wchnschr. 38:265-68, March 15, 1960.

The authors estimated the renal threshold for glucose, the tubular reabsorptive capacity and the glucose excretion after prolonged intravenous glucose load in twenty-one normal individuals. The insulin, creatinine and sodiumthiosulfate clearance tests were used. The glucose load consisted of an intravenous infusion of 20 per cent glucose for a period of three and one-half hours, preceded by an oral priming with 600 cc. fluid (tea). The glucose load caused a gradually increasing glucose excretion and a decrease of the renal threshold for glucose. The decrease of the renal threshold appeared to be due to both an absolute fall of the tubular reabsorptive capacity and a relative fall in its relation to the filtration rate for glucose. The degree of these functional alterations was directly related to the duration of the test period. The authors conclude that the prolonged glucose load leads to an exhaustion of the fermentative processes in the tubules. They suggest that long lasting glycosuria represents a severe stress for the tubular epithelium and may be responsible for the tubular degeneration observed in glycosuric patients. (German) M.G.G.

Munkner, C. (Second Univ. Clin. of Int. Med., Kommunehospitalet, Aarhus, Denmark): CHANGES IN NONESTERIFIED FATTY ACIDS IN HUMAN PLASMA AFTER INGESTION OF VARIOUS FOODSTUFFS. Scandinav. J. Clin. & Lab. Invest. 11: 394-98, 1959.

Oral administration of glucose or protein or fat in amounts as small as 10 gm. is followed by a decrease in the plasma concentration of nonesterified fatty acids (NEFA). The fall depends on the type and amount of the foodstuff administered. The decrease induced by oral administration of oleic acid and animal and vegetable fats is only of short duration. That following ingestion of glucose and protein is longer. Administration of barium sulphate did not result in any change in the plasma concentration of NEFA. G.A.W.

Newey, H.; Parsons, B. J.; and Smyth, D. H. (Dept. of Physiol., University of Sheffield, Sheffield, England): THE SITE OF ACTION OF PHLORHIZIN IN INHIBITING INTESTINAL ABSORPTION OF GLUCOSE. J. Physiol. 148:83-92, October 1959.

Segments of rat intestine were incubated aerobically in solutions containing C^{14}-glucose. The radioactivity of the metabolic carbon dioxide was studied to determine whether glucose had penetrated into the intestinal cells. Even at concentrations which have no effect on endogenous metabolism phlorhizin inhibited the transfer of glucose through the luminal side of the intestinal mucosal cell. G.A.W.

Nikkilä, Esko A.; Jakobson, Theodor; Jokipii, S. G.; and Karlsson, Ka. (First Dept. of of Med., University of Helsinki, and the Municipal Home for Old People, Helsinki, Finland): THYROID FUNCTION IN DIABETIC PATIENTS UNDER LONG-TERM SULFONYLUREA TREATMENT. Acta endocrinol. 33:623-29, April 1960.

Thyroid function was studied in long-term (minimum two years) treatment of diabetes with sulfonylurea drugs, sixty-two with carbutamide and nine with tolbutamide. The tests used were blood PBI, blood cholesterol, and twenty-four-hour uptake of RaI by the thyroid gland. Although the function was normal by all measurements during therapy, in seven patients the uptake of RaI rose sharply within two to seven days after discontinuance of the sulfonylurea therapy. S.B.B.

Oyer, Calvin E. (Metabolic and Endocrine Clinic, Dept. of Internal Med., Saint Michael's Hosp., Newark, N. J.): TOLBUTAMIDE IN TREATMENT OF DIABETES MELLITUS: RESULTS IN PATIENTS FOLLOWED UP TO 17 MONTHS. J. M. Soc. New Jersey 57:117-20, March 1960.

Tolbutamide is a safe, orally administered agent of definite but limited value in the treatment of diabetes mellitus. It seems worthy of trial in diabetic patients past forty years of age with relatively short duration of diabetes and low insulin requirement. The physician must expect a fairly high incidence of failures of treatment, both initially and secondarily. W.R.K.

Page, Otto C.; Garland, James; Stephens, John W.; and Hare, Robert L. (Portland, Oreg.): THE ELECTROCARDIOGRAM IN INFANTS OF DIABETIC MOTHERS. J. Pediat. 56:66-74, January 1960.

The authors report data on forty-six electrocardiograms on twenty-one newborn infants of diabetic mothers taken during the neonatal period. One tracing showed S-T abnormalities during the first day of life and one tracing showed a main T axis of —20 degrees at eight days. A separate description is given of EKG abnormalities in a case of complete transposition of the great vessels. The other EKG's were all within normal limits according to Zigler's criteria. The authors question evidence presented by others said to suggest EKG abnormalities on a basis of hypokalemia secondary to adrenal cortical hyperplasia in infants of diabetic mothers, and suggest the possibility that these abnormalities may be due to exaggeration of the increased load on the left ventricle secondary to circulatory changes following delivery. R.L.J.

Pratt, O. E. (Maudsley Hosp., London S.E.5, England): INDEPENDENCE OF THE HYPERGLYCAEMIC AND THE NITROGEN CATABOLIC EFFECTS OF CORTISONE. J. Physiol. *148*:19P-20P, October 1959.

Cortisone-treated rabbits and rats were given sufficient Protamine Zinc Insulin (10-80 units per day) to avoid hyperglycemia or glycosuria, and determinations were made of blood urea, nitrogen balance, and urinary nitrogen output. Urine and feces were collected and food intake measured for various periods up to thirty-six days treatment with cortisone in doses of up to 50 mg. per kilogram of body weight. All animals given either cortisone or cortisone with insulin showed a negative nitrogen balance after two to six days despite an increased food intake. The nitrogen loss was accounted for mainly by increased urea excretion. The blood urea level was significantly elevated in both the cortisone and the cortisone-insulin groups. Both experimental groups showed sodium retention. It is concluded that cortisone-induced nitrogen loss is due to some defect in nitrogen metabolism and is not merely the result of impaired ability to use carbohydrate. G.A.W.

Raben, M. S.; and Hollenberg, H. C. (The Ziskind Research Lab., New England Center Hosp. & the Dept. of Med., Tufts Univ. Sch. of Med., Boston, Mass.): EFFECT OF GLUCOSE AND INSULIN ON THE ESTERIFICATION OF FATTY ACIDS BY ISOLATED ADIPOSE TISSUE. J. Clin. Invest. 39:435-39, February 1960.

The authors studied the exchange of free fatty acids between rat epididymal adipose tissue and incubating medium. Glucose or insulin alone and especially together promote the disappearance of these acids from the medium. This occurred to a greater degree when oleic acid was added to the medium.

The authors discussed these findings and indicated that they implied that enhancement of esterification of fatty acids may be one mechanism which could explain the reduced mobilization of nonesterified fatty acids which occurs in vivo when carbohydrate utilization is increased. S.B.B.

Robertson, James (Diabetic Clin., Willesden Gen. Hosp., London, England): THE ADMINISTRATIVE ASPECTS OF DIABETES. M. Press *242*:514-17, Dec. 16, 1959.

In this account brief mention has been made of the ways a diabetic can be assisted by the National Health Service and public voluntary organizations. W.R.K.

Rose, Vera (Dept. of Paediatrics, Univ. of Toronto, Toronto, Ont.): INFANTS OF DIABETIC MOTHERS: CLINICAL AND PATHOLOGICAL FEATURES IN A SERIES OF 25 CASES. Canad. M.A.J. *82*:306-10, Feb. 6, 1960.

The author reviewed the clinical and biochemical findings in a group of twenty-five newborn infants of diabetic mothers during the first week of life. There was a mortality rate of 20 per cent among twenty-five infants. Cesarean section was performed seventeen times. Prematurity of the infants was indicated by the presence of immature subcapsular glomeruli in the kidneys at autopsy. Fluid balance studies did not support the view that early weight loss was due to diuresis. Twenty-one infants had electrocardiograms with a variety of alterations, especially a prolonged Q-T interval. Elevated blood potassium, low blood sugar, and low blood calcium levels were found in many infants but with no constant clinical correlation except perhaps for a suggestive relationship between elevated blood potassium and respiratory difficulty.

Assays of urinary excretion of 17-OH corticoids and 17-ketosteroids were performed in seven male infants and no significant deviations from normal found. All five infants who died showed at autopsy hyaline membrane formation in the lungs, increased pancreatic insulin and hypertrophy and hyperplasia of the cells of the islets of Langerhans. S.B.B.

Russek, Henry I. (Staten Island, N. Y.): EMOTIONAL FACTORS IN ATHEROSCLEROSIS. Geriatrics *14*:479-82, August 1959.

The author presents a study of 100 coronary patients under the age of forty and 100 normal controls. He suggests that emotional factors appear to be responsible for disturbance of fat metabolism and blood coagulability and for an increased receptivity of the vascular wall to cholesterol. Emotional stress of occupational origin and combination of a high fat diet seem closely correlated with a staggering incidence of coronary heart disease in the United States. S.S.

Shea, Stephen M.; Robbins, Stanley L.; and Mallory, G. Kenneth (Mallory Inst. of Pathol., Boston City Hosp., Depts. of Pathol., Boston Univ. Sch. of Med. & Harvard Med. Sch., Boston, Mass.): DIABETIC NEPHROPATHY. SAMPLING AND QUANTITATIVE EVALUATION OF AN AUTOPSY POPULATION WITH KIMMELSTIEL-WILSON LESIONS. A. M. A. Arch. Path. 68:447-55, October 1959.

In order to study the relationship between severity of the nodular lesion of intercapillary glomerulosclerosis and severity of renal arteriolar hyalinization on the one hand and the relationship of these lesions to duration and severity of diabetes on the other, a sample of twenty autopsy cases were studied.

There is good correlation between severity of the nodular lesion and extent of renal arteriolar hyalinization. It is unlikely that the latter causes the former since renal arterio-

sclerosis is found to a significant degree in some nondiabetic diseases (e.g., hypertension) without an accompanying nodular lesion. It seems probable that some aspect of diabetes is involved in the production of both the nodular lesion and renal arteriolar hyalinization.

Both the nodular and arteriolar lesions show a weak positive correlation with the duration of diabetes and fail to show any correlation with severity of diabetes as measured by insulin requirement. H.L.W.

Shoemaker, William O.; Mahler, Robert; Ashmore, James; Pugh, Daniel E.; and Hastings, A. Baird (Dept. of Biol. Chem., Harvard Med. Sch., and Dept. of Surg., Peter Bent Brigham Hosp., Boston, Mass.): THE HEPATIC GLUCOSE RESPONSE TO INSULIN IN THE UNANESTHETIZED DOG. J. Biol. Chem. *234*:1631-33, July 1959.

In the first group of experiments, the dog liver was perfused directly by means of cannulas placed in the portal vein and vena cava with oxygenated blood. When 30 to 50 units of glucagon-free insulin were added to the portal venous (inflow) catheter, there was a slow rise in hepatic glucose output. No change in hepatic glycogen occurred following insulin.

The second group of experiments were performed two to eight days after catheters had been placed in the portal, hepatic and splenic arterial vessels. Injection of 0.1 to 0.2 units per kilogram of body weight of glucagon-free insulin into an indifferent artery or vein produced a fall in glucose concentration in plasma from all three sites. Total splanchnic and nonhepatic splanchnic glucose gradients decreased, but the hepatic gradient remained unchanged.

The data showed that insulin failed to alter glucose output by the liver. This is contrary to many other studies in the literature. Insulin instead caused the nonhepatic splanchnic tissues to take up glucose. A.R.C., JR.

Smith, Marthe E.; Burnham, DeWitt K.; and Black, Melvin B. (Dept. of Path. and Div. Med., St. Luke's Hosp., San Francisco, Calif.): CEREBRAL MUCORMYCOSIS: REPORT OF A CASE. A. M. A. Arch. Path. 66:468-73, October 1958.

A case of fatal cerebral mucormycosis is presented. Since the few reported cases have had rather stereotyped manifestations, more widespread knowledge of this condition should result in more frequent antemortem diagnoses and more thorough investigation at autopsy. It is suggested that routine examination of paranasal sinuses in diabetics coming to autopsy might help elucidate the pathogenesis. W.R.K.

Stadler, G.; Schmid, R.; and Wolff, M.v. (Universitäts-Kinderklinik, Basel, Switzerland): FUNCTIONAL RENAL MICRO-ANGIOPATHY IN INSULIN-TREATED DIABETIC CHILDREN. Deutsche med. Wchnschr. *85*:346-50, Feb. 26, 1960.

Renal function tests were carried out in eighteen diabetic children (aged four to eighteen years) requiring insulin. None of the patients had clinical evidence of renal disease and only one had retinopathy. It was found that marked changes in renal function were demonstrable within a few weeks after the first recognition of the diabetes. These manifested themselves in increases of the insulin-clearance and of the filtration fraction with an elevated $TmPO_4$ and Tm glucose and a highly labile and variable renal blood flow (PAH clearance). The authors describe these changes as "functional micro-angiopathies" and suggest that the abnormal renal blood flow causes unphysiologic variations in pressure and particularly an increased pressure in the glomerular capillaries; these become more permeable as result of mechanical overstretching. Persisting or recurring "functional micro-angiopathies" may ultimately result in the typical lesions of the intercapillary glomerulosclerosis, which is found so frequently in the adult diabetic. M.G.G.

Stuart, Robert C.; Piazza, Eugene U.; Hyman, Harris, III; and Hurwitz, David: CHLORPROPAMIDE THERAPY OF DIABETES. AN APPRAISAL. New England J. Med. *261*:427-30, Aug. 27, 1959.

This deals with the use of chlorpropamide in fifty diabetic patients treated in an outpatient clinic. The drug was found to be effective in 54 per cent of the cases of adult onset diabetes. Excellent control was observed in 38 per cent of the patients, greater than that observed when the same patients were on insulin or tolbutamide. Quite severe toxic side effects occurred in four cases, two of these consisting of exfoliative dermatitis and jaundice. An additional group of fifty patients on chlorpropamide for at least two months showed no toxic reactions. Chlorpropamide has proved to be an effective hypoglycemic agent. Further evaluation of toxicity in long-term effectiveness of this drug is necessary. S.S.

Summ, H. D.; Creutzfeldt, W.; and Wallenfels, K. (Depts. of Organic Chemistry and of Medicine, Universität Freiburg i. Br., Germany): ON THE INFLUENCE OF TOLBUTAMIDE (D-860) AND OF INSULIN ON $^{14}CO_2$-FIXATION IN LIVER GLYCOGEN. Klin. Wchnschr. *38*:85-87, Jan. 15, 1960.

Groups of five albino rats were fasted for eighteen hours and then given an intraperitoneal injection of 50 μc $NaHC^{14}O_3$/kg. Two groups served as controls; one group received in addition a subcutaneous injection of 5 units regular insulin/kg. and two other groups received instead of insulin, oral medication of tolbutamide in doses of 250 mg./kg. and 500 mg./kg. respectively. The animals were sacrificed after three hours, the liver glycogen was determined photometrically, and C^{14} activity of the glycogen isolated from the pooled five livers of each group was estimated. The C^{14} activity of the isolated liver glycogen was the highest in the tolbutamide treated group, the lowest in the insulin treated group. Compared with the untreated control groups tolbutamide appeared to increase, insulin to decrease the C^{14} activity. These results suggest that tolbutamide causes in the liver both an increased gluconeogenesis and an accelerated glycogen turnover, while insulin decreases the glycogen turnover. The authors assume that the sulfonylureas might have a similar activating effect upon the glucose metabolism in the pancreatic beta cells and that this phenomenon may be responsible for the increased insulin release from the beta cells observed after sulfonylurea medication. M.G.G.

Takos, Michael J.; and Menzin, A. William (Dade County Dept. Public Health, Miami, Fla.): THE RELATIONSHIP OF TETANUS TO CHRONIC SKIN ULCERS AND DIABETES MELLITUS. J. Chron. Dis. *11*:170-75, February 1960.

A group of 170 patients who had developed tetanus over a period of fourteen years were analyzed. Among these were patients who had skin ulceration of the exposed parts as the portal of entry. Diabetics appeared to be more frequently affected than their incidence in the population. Among these were some (including the diabetics) without overt skin ulceration. The authors suggest tetanus immunization and re-immunization every three years of patients with chronic skin ulcers to prevent this complication. S.B.B.

Thompson, Marilyn M.; and Mayer, Jean (Dept. of Nutrition, Harvard Sch. of Pub. Health, Boston, Mass.): HYPOGLYCEMIC EFFECTS OF SACCHARIN IN EXPERIMENTAL ANIMALS. Am. J. Clin. Nutrition 7:80-85, January-February 1959.

It has been known in the past that saccharin has a hypoglycemic effect on animals. The authors did both acute and chronic experiments, using obese-hyperglycemic mice, lean littermates of these mice, and adult female Wistar rats.

In the acute experiment, which ran for four weeks, the administration of the saccharin solution was made both by intraperitoneal injection and by stomach tube, each rat receiving 100 mg. of saccharin. In this group of rats hypoglycemia was produced in the obese-hyperglycemic mice but not in the other animals. The authors theorize that the possible mechanism for the hypoglycemic effect of saccharin may be similar to that of the sulfonylureas and sulfonamides in general, although they have demonstrated that carbutamide is ineffective in reducing blood sugar of the obese-hyperglycemic mice, which suggests that saccharin may act by a selective and unique mechanism of its own. R.C.C.

Traisman, Howard S.; and Newcomb, Alvah L. (Children's Memorial Hosp. & Northwestern Med. Sch., Chicago; Evanston Hosp., Evanston, Ill.): CLINICAL USE OF GLUCAGON IN JUVENILE DIABETES MELLITUS. Illinois M. J. 117:81-82, February 1960.

The prompt significant rise in blood sugar following administration of parenteral glucagon is described in four patients. Relief from the symptoms of insulin-induced hypoglycemia in juvenile diabetic patients occurred after intramuscular administration of glucagon (0.50-0.10 mg. per kilogram of body weight). Hypoglycemia in a newborn infant of a diabetic mother was also successfully treated in this manner.

Glucagon effectively relieves hypoglycemia which is severe enough to preclude oral feeding in selected diabetic children, and may also prove beneficial to the hypoglycemic infants of diabetic mothers. H.L.W.

Warren, K. W.; and Cattell, R. B. (Lahey Clin., Boston, Mass.): PANCREATIC SURGERY. New England J. Med. 261: 280-87, 333-39, 387-92, Aug. 6, 13, 20, 1959.

A comprehensive review involving pancreatic surgery dealing with the problems of acute pancreatitis, postoperative pancreatitis, chronic relapsing pancreatitis, pancreatolithiasis, pancreatic cysts and islet cell adenomas and hyperinsulinism. Surgical experiences involving carcinoma of the pancreas and periampullary area are also comprehensively covered. S.S.

Williams, Robert H.; Pollen, Richard H.; Tanner, Donald C.; and Barnes, Robert H. (Dept. of Med., Univ. of Washington Sch. of Med., Seattle, Wash.): ORAL ANTIDIABETIC THERAPY. Ann. Int. Med. 51:1121-33, December 1959.

The authors review the mechanism and clinical aspects of oral hypoglycemic therapy with tolbutamide, chlorpropamide, metahexamide and DBI. They draw upon their own clinical experience as well as give us some details of hitherto unpublished surveys by medical directors of the pharmaceutical firms which had sponsored the clinical testing of these drugs. S.B.B.

Winegrad, Albert I.; Shaw, Walter N.; Lukens, Francis D. W.; Stadie, William C.; and Renold, Albert E. (George S. Cox Med. Res. Inst. and John Herr Musser Dept. of Res. Med., Univ. of Penn., and Dept. of Med., Harvard Med. Sch., Boston, Mass.): EFFECTS OF GROWTH HORMONE IN VITRO ON THE METABOLISM OF GLUCOSE IN RAT ADIPOSE TISSUE. J. Biol. Chem. 234:1922-28, July 1959.

Bovine growth hormone administered either to normal fed or to alloxan diabetic rats produced an increase in the oxidation of glucose carbon to CO_2 in paired epididymal fat pads. In neither type of animal was the long-chain fatty acid synthesis influenced by growth hormone. The hormone stimulated oxidation of carbon 1 and carbon 6 in adipose tissue, particularly the latter. Addition of insulin further enhanced CO_2 production, especially from carbon 1, and long-chain fatty acid synthesis was also increased. The effects of growth hormone could not be reproduced by insulin, and the two substances operate in different manners. A.R.C., JR.

Wittels, Benjamin, and Reiner, Leopold (Bronx Hosp., & Albert Einstein Coll. of Med., New York, N. Y.): HISTOCHEMICAL OBSERVATIONS ON GLYCOGEN IN THE HUMAN MYOCARDIUM. Am. J. Path. 36:55-75, January 1960.

Stained glycogen was studied in the postmortem myocardium of forty-one adults. The persistence of glycogen was associated with myocardial infarction though no glycogen was found in the infarction itself. Postmortem interval, function of fibers, nutritional status, diabetes mellitus, pulmonary disorder, congestive heart failure, hyperpotassemia, and cardiac glycosides had no independent effect on the persistence of glycogen.

Glycogen persisted throughout the left ventricle after a fresh infarction but was limited to a narrow border around the healing infarction. The persistence of glycogen is thought to be a manifestation of metabolic damage to the muscle cell. H.L.W.

Wolf, Helmut (Dept. of Pediatrics, University of Göttingen, Germany): THE BLOOD SUGAR LEVEL IN THE NEW-BORN. Klin. Wchnschr. 38:87-89, Jan. 15, 1960.

The known fluctuations of blood sugar level during the first hours and days of life were re-examined, using the glucose-oxidase method. In twenty cases, blood samples were taken from the cord immediately after delivery and from the fingertip of the newborn 1, 6, 12, 24, 48, 72, 96 and 144 hours later. The values were compared with the blood sugar level of the mothers which was found to average 87 $\pm$ 4 mg. per 100 ml. The average cord blood sugar was significantly lower with 67 $\pm$ 4 mg. per 100 ml. The one-hour blood sugar level of the newborn was markedly lower with 26 $\pm$ 3 mg. per 100 ml., but with a wide range from 8 to 81 mg. per 100 ml. During the subsequent hours, there was a very slow rise of the blood sugar level of the newborn, but at the end of six days it was still significantly lower than that of the mothers and averaged 45 $\pm$ 3 mg. per 100 ml. (German) M.G.G.

Wong, Rose K. L.; and Van Bruggen, J. T. (Dept. of Biochemistry, Univ. of Oregon Med. Sch., Portland, Oreg.): LIPID METABOLISM IN THE DIABETIC RAT. I. ACETATE METABOLISM AND LIPID SYNTHESIS IN VIVO. J. Biol. Chem. 235:26-29, January 1960.

Hypercholesterolemia amounting to 64 per cent more than in a control group was found in untreated alloxan or pancreatectomized diabetic rats. This was not due to increased cholesterol synthesis. Production of CO_2 from acetate-1-C^{14} was normal but alloxanized animals exhibited increased incorporation of C^{14} in CO_2. This supports other studies showing reduced fatty acid synthesis in diabetic preparations. A.R.C., JR.

ORGANIZATION SECTION

OFFICERS AND MEMBERS OF COUNCIL, AMERICAN DIABETES ASSOCIATION, 1960-1961

HONORARY PRESIDENTS, ELLIOTT P. JOSLIN, M.D., *Boston;* CHARLES H. BEST, M.D., *Toronto*

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FRANKLIN B. PECK, SR., M.D., *Indianapolis*

FIRST VICE PRESIDENT
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TREASURER
THOMAS P. SHARKEY, M.D., *Dayton*

EXECUTIVE DIRECTOR
J. RICHARD CONNELLY, *New York*

MEMBERS OF COUNCIL

TERM EXPIRING 1961

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ARTHUR R. COLWELL, SR., M.D., *Chicago*
JOSEPH H. CRAMPTON, M.D., *Seattle*
LAURANCE W. KINSELL, M.D., *Oakland*
RACHMIEL LEVINE, M.D., *Chicago*
KELLY M. WEST, M.D., *Oklahoma City*

TERM EXPIRING 1962

LOUIS K. ALPERT, M.D., *Washington, D.C.*
EDWIN W. GATES, M.D., *Niagara Falls*
HARVEY C. KNOWLES, JR., M.D., *Cincinnati*
ARNOLD LAZAROW, M.D., *Minneapolis*
ALBERT E. RENOLD, M.D., *Boston*
LAURENTIUS O. UNDERDAHL, M.D., *Rochester, Minnesota*

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PRISCILLA WHITE, M.D., *Boston*

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ALEXANDER MARBLE, M.D., *Boston*; FRANCIS D. W. LUKENS, M.D., *Philadelphia*

EX OFFICIO

JOSEPH L. IZZO, M.D., *Rochester, New York,* Chairman, Board of State Governors
MAURICE PROTAS, M.D., *Washington, D.C.,* Chairman, Assembly of Delegates

ELECT OFFICERS AND COUNCILORS AT 20th ANNUAL MEETING

The Twentieth Annual Meeting of the American Diabetes Association was held in Miami Beach June 11-12, 1960. At the Business Session on June 12, Franklin B. Peck, Sr., M.D., Indianapolis, was elected President for this organizational year to succeed Francis D. W. Lukens, M.D., Philadelphia.

Charles H. Best, M.D., Toronto, co-discoverer of insulin, President of the American Diabetes Association in 1948-49 and Banting Memorial Lecturer in 1952, was elected Honorary President. He will serve in this capacity along with Elliott P. Joslin, M.D., Boston, who is also Honorary President.

Blair Holcomb, M.D., Portland, Oregon, was elected First Vice President, and Jerome W. Conn, M.D., Ann Arbor, Second Vice President. E. Paul Sheridan, M.D., Denver, and Thomas P. Sharkey, M.D., Dayton, were re-elected Secretary and Treasurer respectively. Dr. Peck served in 1959-60 as First Vice President while Dr. Holcomb was Second Vice President, and Dr. Conn a Member of the Council (term expiring 1962). All the officers will serve through the Twenty-first Annual Meeting of the Association which will be held in New York City, June 24-25, 1961, at the Commodore Hotel.

At this Business Session, Thaddeus S. Danowski, M.D., Pittsburgh; William H. Grishaw, M.D., Beverly Hills; George J. Hamwi, M.D., Columbus, Ohio; Henry E. Marks, M.D., New York; Henry E. Oppenheimer, M.D., St. Louis; and Priscilla White, M.D., Boston, were elected to the Council for the three-year term expiring in 1963. Albert E. Renold, M.D., Boston, was elected a Member of the Council to fill a term expiring in June 1962.

At the annual sessions of the Board of State Governors and the Assembly of Delegates, Joseph L. Izzo, M.D., Rochester, New York, and Maurice Protas, M.D., Washington, D.C., were re-elected Chairman of each group respectively. Drs. Izzo and Protas therefore will continue to serve as ex officio Members of the Council.

20th ANNUAL MEETING REGISTRATION EXCEEDS 500

Attendance at the Twentieth Annual Meeting at Miami Beach passed the 500 mark, but was approximately 30 per cent less than registration at the previous Annual Meeting held in Atlantic City in 1959. This lowered attendance reflected the experience of the American Medical Association whose annual session in the same city attracted 60 per cent of the anticipated registrants. Attendance at the Miami Beach meeting of the ADA and a comparison with previous years are revealed by the following figures.

		Association Members	Guest Physicians	Other Guests	Total
1960	Miami Beach	282	165	57	504
1959	Atlantic City	404	254	54	712
1958	San Francisco	267	165	27	459
1957	New York	493	182	43	718
1956	Chicago	406	130	29	565
1955	Atlantic City	408	137	35	580
1954	San Francisco	199	99	16	314
1953	New York	438	198	7	643
1952	Chicago	325	102	5	432
1951	Atlantic City	342	125	10	477
1950	San Francisco	162	83	6	251

Detailed information concerning the Banquet of the Twentieth Annual Meeting and reports presented at the Business Session will be published in the Sept.-Oct. issue of DIABETES.

9th POSTGRADUATE COURSE WILL BE HELD IN NEW ORLEANS

As previously announced, the American Diabetes Association will hold its Ninth Postgraduate Course in Diabetes and Basic Metabolic Problems in New Orleans, Jan. 18, 19 and 20, 1961. The Louisiana State University School of Medicine will be the site of the Scientific Program, and the Jung Hotel will serve as headquarters.

The tentative program includes the following subjects: "Enzyme Actions in Carbohydrate Metabolism"; "Carbohydrate, Fat and Protein Metabolism in Liver and Muscles"; "Regulation of Insulin Secretion and Effects"; "Intermediary Metabolism in Diabetes and Other Endocrinopathies"; "A Concept of the Diabetogenic Period in Man with Study of One Parameter"; "Reproduction in Diabetes"; "New and Long-standing Juvenile Diabetes"; "Diabetes as an Added Problem in Medical and Surgical Practice"; "Infections of the Genitourinary Tract in Diabetes"; "Why Is Diabetic Acidosis Still Fatal?"; "Interpretation of Laboratory Tests"; "Diet, Insulin and Oral Hypoglycemic Agents: Comparisons and Contrasts"; "Is There a Place to Combine Insulin and Oral Hypoglycemic Therapy?"; "Resume of Experience with Phenformin (DBI)"; "Syndromes Related to Diabetes Mellitus" ("Hypoglycemias," "Angiopathies," "Nephropathies," "Neuropathies").

The subject of "lipids" will be presented in depth on Wednesday, January 18, with Faculty Members drawn primarily from the Metabolism Study Section of the National Institutes of Health, which will meet in New Orleans January 14-17. "Clinical Aspects of Lipids" will be the title of the day's program.

"Diabetes as a Biological Spectrum," a panel presentation, will be held at the close of the Thursday morning session to discuss the pathogenesis and manifestations of diabetes, and the pathophysiology of the diabetic state. Panelists will be the same morning's Faculty Members. At the close of the Thursday afternoon session, "Seminars on Current Problems in Diabetes" will be held, with each Thursday Faculty Member serving as one of the leaders, using the title of his lecture as the subject of his small seminar group.

Registration Fees are $40 for Association members and $75 for nonmembers. All inquiries pertaining to the Course should be directed to the national office, 1 East 45th Street, New York 17, N. Y.

1961 LILLY AWARD

The fifth Lilly Award will be given at the Twenty-first Annual Meeting of the American Diabetes Association June 24-25, 1961, in New York City. This annual award is supported by Eli Lilly and Company and consists of $1,000 and a medal. The following stipulations govern the contest.

Purpose: To recognize demonstrated research in the field of diabetes, taking into consideration independence of thought and originality.

Eligibility: Any investigator in an appropriate field of work closely related to diabetes who is less than forty years of age on January 1 of the year in which the award is made. The research will not necessarily be judged in comparison to the work of more mature and experienced workers. The candidate should be a resident of the United States or Canada.

Nominations: Nominations for the award will be solicited from the members of the American Diabetes Association. Such nominations will be requested by repeated notices to be published in DIABETES. Names of nominees will be sent to the Chairman of the Committee on Scientific Awards and must be received before December 1 of the year preceding the award. The nomination should be accompanied by full information concerning the nominee's personality, training and research work. Six copies of each item should be submitted. No member may send in more than one nomination. A list of the nominee's publications, if any, and six copies of the publication or manuscript for which the award is to be given should also accompany the nomination. At the discretion of the Committee on Scientific Awards, the award may be given for work published during the year prior to December 1 of the year preceding the award. The nominee should be actively engaged at that time in the line of research for which the award is to be made.

Announcement: The name of the winner will be announced in the program of the Annual Meeting of the Association, and the award presented at that meeting. The winner, subject to the approval of the Committee on Scientific Programs, will be invited to present a paper on his work. Papers considered for the award must be submitted with the idea that they will be published in whole or in part in DIABETES if found acceptable to the Editor and/or Editorial Board. If the Committee should decide that no outstanding work has been presented for this consideration, the award will not be made.

Award: In addition to the monetary award and medal, traveling expenses will be given to make it possible for the recipient to receive his award in person at the Annual Meeting.

ADA RESEARCH FELLOWSHIPS

Three Research Fellowships for the academic year July 1, 1961—June 30, 1962, will be awarded by the Committee on Research and Fellowships. Deadline for applications is Nov. 15, 1960. Requests for application forms and other inquiries should be addressed to Mr. J. Richard Connelly, Executive Director, who will forward the information to the Committee.

"EXERCISE, CALORIES, AND DIABETES" IN ADA *FORECAST*

In response to many requests, an article dealing with exercise for diabetics was published in the July-August 1960 issue of FORECAST. Harvey C. Knowles, Jr., M.D., of Cincinnati was the author. Physicians and others may wish to recommend this article to their diabetic patients and friends. Copies of the July-August issue may be obtained at 35¢ each or six for $2.00.

NOVEMBER 13-19: THE DATE OF DIABETES WEEK

Affiliates of the American Diabetes Association, County and State Medical Societies and other cooperating agencies are urged to begin planning their Public Education and Detection programs now for Diabetes Week November 13-19, which traditionally is observed the week before Thanksgiving.

Joseph H. Crampton, M.D., Seattle, is the newly appointed Chairman of the Committee on Public Education and Detection, succeeding Louis K. Alpert, M.D., of Washington, D.C., who served in that position for six years. Dr. Alpert will continue in the capacity of a Vice Chairman of this Committee.

Material for Diabetes Week is currently being prepared and mailings will go forward in late summer for local use. New this year is an informational pamphlet for service clubs who wish to participate in the Diabetes Detection Drive. Address your requests for information concerning available services and supplies to Mr. J. Richard Connelly, Executive Director, American Diabetes Association, Inc., 1 East 45th Street, New York 17, N. Y.

IDF WILL HOLD 4th CONGRESS

Geneva, Switzerland, will be the site of the Fourth Congress of the International Diabetes Federation. Dates for the Congress are July 10-14, 1961. The ADA Advisory Committee for the program includes Howard F. Root, M.D., Boston, Chairman; Alexander Marble, M.D., Boston; and Franklin B. Peck, Sr., M.D., Indianapolis. The Journal will publish further information about the Congress as it becomes available. Those interested in group transportation should communicate as soon as possible with Howard F. Root, M.D., 15 Joslin Road, Boston 15, Mass.

NEW MEMBERS

The following were elected as of June 1 and July 1, 1960.

Active

California

Gattas, Fred A.	Ontario
Glaubach, Nathan	Los Angeles
Tucker, Alvin Z.	San Bernardino

Georgia

Hertell, Joseph A.	Atlanta

Kansas

Svoboda, Charles R.	Chapman

New Jersey

Finkel, Marion J.	Rahway

New Mexico

Basile, Evelyn J.	Las Cruces

New York

Blauner, Stuart S.	White Plains

Ohio

Skillman, Thomas G.	Columbus

Oregon

Quinn, Edwin	Coos Bay

Pennsylvania

Bendersky, Gordon	Philadelphia
Carruthers, Bruce M.	Philadelphia
Faludi-Siegler, Georgina	Philadelphia
Klitch, George M.	Harrisburg

Washington

Nielsen, Robert L.	Seattle

Other Countries

Philippines

Santiago, Nestor M.	Manila

NEWS OF AFFILIATE ASSOCIATIONS

The WASHINGTON DIABETES ASSOCIATION held its Sixth Annual Symposium on Metabolic and Endocrine Diseases on April 23, 1960, at the University of Washington in Seattle. The following program was presented:

Introduction, by Donald C. Tanner, M.D., Seattle; "Polyunsaturated Fat Diets in Diabetes," by Laurance W. Kinsell, M.D., San Francisco; "The Role of Aldosterone in Clinical Disease," by Frederic C. Bartter, M.D., Bethesda; panel discussion entitled "The Syndrome(s) of Edema in Women," by Drs. Bartter, Kinsell and Robert L. Nielsen, Seattle, moderated by John L. Bakke, M.D., Seattle. C. Alvin Paulsen, M.D., Seattle, was moderator for "Lipid Metabolism in the Diabetic Subject as Studied with the Aid of C^{14}-labeled Lipids and Lipid Precursors," by Dr. Kinsell; and "Hyponatremia," by Dr. Bartter. A General Discussion was moderated by Joseph H. Crampton, M.D., Seattle.

NEWS NOTES

AMERICAN COLLEGE OF PHYSICIANS OFFERS POSTGRADUATE COURSES

The Autumn-Winter program of postgraduate courses of The American College of Physicians for the 1960-61 season will include the following: "Hematology and Radioisotopes," at The Ohio State University College of Medicine, Columbus, Ohio, Sept. 19-23, 1960; "Cancer and the Internist—1960 Concepts," at Memorial Center, Sloan-Kettering Institute for Cancer Research, New York City, Oct. 10-14, 1960; "Electrocardiography," at the University of Utah College of Medicine, Salt Lake City, Utah, Nov. 7-11, 1960; "Recent Advances in Drug Therapy," at the University of Washington School of Medicine, Seattle, Robert H. Williams, M.D., Director, Jan. 9-13, 1961; "Mechanisms of Disease," at Columbia University College of Physicians and Surgeons, Presbyterian Hospital, New York City, Jan. 16-20, 1961; "Selected Topics in Internal Medicine," at The University of Oklahoma School of Medicine and University Hospitals, Oklahoma City, Feb. 20-24, 1961.

Members of the American Diabetes Association who participated in the Forty-first Annual Session of The American College of Physicians at San Francisco, April 4-8, 1960, included the following:

April 4

Garfield G. Duncan, M.D., Philadelphia, and Dwight L. Wilbur, M.D., San Francisco, took part in a panel discussion on "The Health Problems of the American Executive." Dr. Duncan spoke on "Overweight, Overstress and High Blood Pressure," and Dr. Wilbur on "Formula for a Productive and Happy Retirement."

Garfield G. Duncan, M.D., Philadelphia, Presiding Officer for afternoon Clinical Session.

Robert H. Williams, M.D., Seattle, Presiding Officer of Basic Medical Science Session, "Microbiology; Cardiology."

Stanton Segal, M.D., Bethesda, "Progesterone Effects on Galactose Metabolism."

J. Kent, M.D., San Francisco, and Peter H. Forsham, M.D., San Francisco, "Clinical Use of a New Diagnostic Agent (SU-4885) in the Recognition of Pituitary and Adrenocortical Disorders."

April 5

Alfred W. Harris, M.D., Dallas, "Clinic on Treatment of Recurrent Cardiac Arrhythmias."

Ralph O. Wallerstein, M.D., San Francisco, "Diagnosis and Treatment of Hematologic Disease."

James J. McGinnis, M.D., San Francisco, and John M. Elliott, M.D., San Francisco, "Unusual Causes of Hypertension and Their Treatments."

G. B. Robson, M.D., San Francisco, "Cardiac-Surgical Conference."

Campbell Moses, Jr., M.D., Pittsburgh, "Lipid Metabolism and Arteriosclerosis."

Dwight L. Wilbur, M.D., San Francisco, Presiding Officer Clinical Session.

Walter de M. Scriver, M.D., Montreal, Presiding Officer Clinical Session.

Robert H. Williams, M.D., Seattle, Presiding Officer Basic Medical Science Session, "Life and Its Course."

Hyman J. Zimmerman, M.D., Chicago, "The Clinical Significance of Plasma and Erythrocyte Enzymes in Anemia."

William E. Connor, M.D., Iowa City, "The Antithrombotic Properties of Coumarin Drugs."

April 6

Robert H. Williams, M.D., Seattle, Moderator, Garfield G. Duncan, M.D., Philadelphia, Stefan S. Fajans, M.D., Ann Arbor, Raymond V. Randall, M.D., Rochester, Minnesota: Panel "Use of Hypoglycemic Agents."

Samuel G. Taylor, III, M.D., Chicago, "Chemotherapy of Cancer."

Eunice M. Lasché, M.D., Tampa, Florida, "A Clinical Study of the Chronic Lung Disease Due to Nonchromogenic Acid-Fast Organisms."

Robert H. Williams, M.D., Seattle, Presiding Officer Basic Medical Science Session "Fat Metabolism."

Marvin D. Siperstein, M.D., Dallas, "Lipogenesis and Cholesterologenesis."

Laurance W. Kinsell, M.D., San Francisco, "Effect of Diet on Lipids in Plasma and Depots."

April 7

Peter H. Forsham, M.D., San Francisco, Moderator, Clinic on "The Diagnosis and Treatment of Diseases of the Adrenals."

James J. McGinnis, M.D., San Francisco, Clinic on "Neurological Problems of Interest to the Internist."

DeWitt K. Burnham, M.D., San Francisco, Moderator, G. B. Robson, M.D., San Francisco, Garfield G. Duncan, M.D., Philadelphia, Stefan S. Fajans, M.D., Ann Arbor, Leo P. Krall, M.D., Boston: "A Panel of Experts Meet and Treat Our Diabetic Patients."

Felix O. Kolb, M.D., San Francisco, Moderator, "Medical Management of Renal Stones."

Robert H. Williams, M.D., Seattle, and Wm. W. H. Pote, Jr., M.D., Los Angeles, "The Newer Oral Diabetic Agents."

Robert H. Williams, M.D., Seattle, Presiding Officer Basic Medical Science Session "Hormones; Metabolism; Submicroscopic Structure."

April 8

Peter H. Forsham, M.D., San Francisco, Color Television of Medical Clinics, "Arterial Hypertension—Curable Secondary Hypertension."

John C. Beck, M.D., Montreal, and J. Lester Gabrilove, M.D., New York City, panel on "Adrenal Cortical Hyperfunction."

George W. Thorn, M.D., Boston, "ACTH-secreting Pituitary Tumors."

Clifford F. Gastineau, M.D., Rochester, Minnesota, "The Occurrence of Clinical Disorders of the Thyroid in Patients with Addison's Disease."

PERSONALS

RACHMIEL LEVINE, M.D., has been appointed Professor and Chairman of the Department of Medicine at New York Medical College. He is currently Professor of Medicine at Chicago Medical School, Professorial Lecturer in Physiology at the University of Chicago, and Chairman of the Department of Medicine and Director of Medical Education at Michael Reese Medical Center. Dr. Levine is scheduled to assume his new position in the fall.

At New York Medical College, Dr. Levine also will be director of the Department of Medicine at the College's Flower and Fifth Avenue Hospitals, and Metropolitan Hospital. He expects to set up a broad program for investigation in diabetes. Dr. Levine is a Councilor of the American Diabetes Association, and Chairman of the Committee on Oral Hypoglycemic Compounds.

BEVERLY C. SMITH, M.D., was co-chairman of the Scientific Exhibits Subcommittee of the 154th Annual Meeting of the Medical Society of the State of New York in New York City May 7-13, 1960. SEYMOUR K. FINEBERG, M.D., was in charge of the exhibit on "Management of the Obese Diabetic." KERMIT E. OSSERMAN, M.D., was one of several in charge of the exhibit on "Myasthenia Gravis."

NECROLOGY

MORRIS ANT, New York, New York, born December 9, 1894.
WILLIAM M. DUGAN, Indianapolis, Indiana, born July 11, 1904.